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CLINICAL APPLICATION OF A NEW TEST OF PITUITARY RESERVE*

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ABSTRACT

SU-4885 inhibits 11β-hydroxylation of steroids by the human adrenal cortex and leads to a decrease in cortisol secretion, a "compensatory" rise in ACTH secretion, and the secretion of large quantities of 11-desoxycorticosteroids such as compound S. Compound S and its metabolites are readily measurable in biologic fluids as 17-hydroxycorticoids. In normal subjects, therefore, SU-4885 induces a rise in total blood and urinary 17-hydroxycorticoids. Since this response to SU-4885 is dependent upon a rise in ACTH secretion it affords, in patients who do not have frank adrenal insufficiency, a sensitive means of testing the reserve capacity of the pituitary gland to secrete ACTH. Of 9 patients with chromophobe adenomas tested in this manner, 6 were found to have "limited pituitary reserve." This was true also of 2 out of 5 patients with acromegaly, 3 out of 6 patients with cachexia, and 1 patient with post-traumatic diabetes insipidus. Of 7 patients with active Cushing's disease due to adrenal hyperplasia, all exhibited responses which were greater than the average response of normal subjects. Of 4 patients who had been successfully treated for Cushing's disease by pituitary irradiation, all exhibited subnormal responses to SU-4885. Of 3 patients with Cushing's syndrome secondary to adrenal tumor, none exhibited increases in 17-hydroxycorticoid excretion in response to SU-

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4885. Of 6 patients with myxedema due to primary hypothyroidism all responded normally. It is concluded that SU-4885 affords a sensitive means of testing the responsiveness of the ACTH-secreting mechanism in various clinical situations.

INTRODUCTION

IN A recent report (1) a new approach to the evaluation of pituitary reserve was proposed, based upon the theory that a reduction in the secretion of cortisol by the adrenal cortex induces a compensatory increase in the secretion of adrenocorticotropin (ACTH) by the pituitary. An agent which impairs the synthesis of cortisol (1–3) is 2-methyl-1,2-bis(3-pyridyl)-1-propanone (SU-4885), (Fig. 1); in low concentrations it is a relatively selective inhibitor of 11β-hydroxylation. In consequence of this inhibition, 11-desoxycortisol (compound S) tends to replace cortisol in the adrenal effluent. Compound S is not an effective suppressor of ACTH secretion; thus, as the level of cortisol falls there is an increase in the pituitary secretion of ACTH. The increase in ACTH secretion results in a large increase

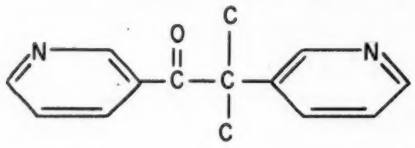

FIG. 1. Structural formula of 2-methyl-1,2-bis-(3-pyridyl)-1-propanone or SU-4885.

SU - 4885

in the adrenocortical secretion of 11-desoxycorticosteroids, so that the quantity of compound S secreted usually far exceeds the original quantity of cortisol. Like cortisol, compound S and its ring A-reduced metabolite, tetrahydro-compound S, give positive reactions in the Porter-Silber test for 17,21-dihydroxy-20-ketosteroids ("17-hydroxycorticoids"). Following treatment with SU-4885, *total* 17-hydroxycorticoid levels in blood and urine are increased, and the *increases* are due entirely to compound S and its metabolites. The rise in total 17-hydroxycorticoid secretion which occurs following treatment with SU-4885 is dependent upon the functional integrity of both the adenohypophysis and the adrenal cortex. The possible diagnostic use of this phenomenon in assessing pituitary-adrenal integrity is explored in the present study.

METHODS

Selected for this study were 71 subjects whose endocrinologic status had been clearly defined by conventional methods. In most instances SU-4885 was administered orally in dosages of 500 mg. or 750 mg. every four hours for 6 doses. In order to prevent the transient vertigo which may occur after rapid absorption (or rapid injection) of SU-4885,

each oral dose was administered following a glass of milk or some solid food. In certain studies SU-4885 ditartrate was administered intravenously as a slow constant infusion over a period of sixteen hours; the total dose of 2 or 3 Gm. of the salt was diluted in 1 liter of physiologic sodium chloride or 5 per cent dextrose in water. No side-effects were observed during such infusions. Except when specifically mentioned (under Results and Comments), treatment with exogenous ACTH or with steroids capable of suppressing endogenous ACTH was carefully avoided for at least three days prior to, and one day following treatment with SU-4885. "Standard ACTH tests" were performed by the continuous intravenous infusion of 50 units of corticotropin over a period of exactly eight hours. Complete 24-hour collections of urine were made throughout each study, and were preserved with thymol and acetic acid. Total urinary 17-hydroxycorticoids and free plasma 17-hydroxycorticoids were determined by modifications of the method of Silber and Porter (4). Urinary 17-ketosteroids were determined by a modification of the method of Callow, Callow and Emmens (5). (According to these procedures as performed in this laboratory, in 98 per cent of normal adults the 24-hour urinary excretion of 17-hydroxycorticoids is within the range of 3 to 12 mg. under "basal" conditions, and 15 to 45 mg. in response to "standard ACTH tests.")

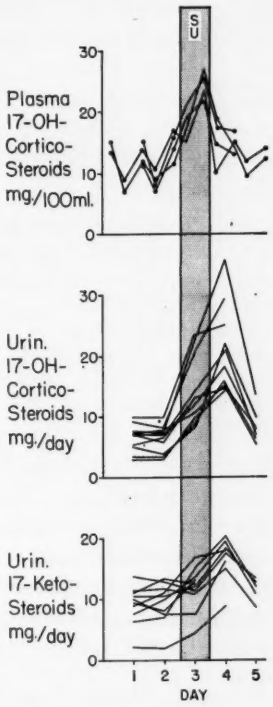

FIG. 2. The normal response to SU-4885. Normal adults receiving SU-4885 for twenty-four hours exhibited increases in the level of plasma 17-hydroxycorticoids, urinary 17-hydroxycorticoids and urinary 17-ketosteroids. Peak values were usually observed on the day following treatment.

RESULTS AND COMMENTS

Normal adults

Eleven endocrinologically normal subjects were given one-day courses of treatment with SU-4885. The effects on plasma and urinary steroid values are summarized in Figure 2. In all subjects, increases in urinary 17-hydroxycorticoids occurred during the day of treatment and further increases during the 24-hour period following cessation of treatment. Normal values were usually observed again on the second day following treatment.

Urinary 17-ketosteroid values rose and fell simultaneously with 17-hydroxycorticoid values, but the changes were not so great as in the case of 17-hydroxycorticoids. This was true not only in normal subjects but in all of the various other groups studied. Because the 17-ketosteroid data have the same significance as the 17-hydroxycorticoid data insofar as the present study is concerned, they will not be reported in detail in this paper.

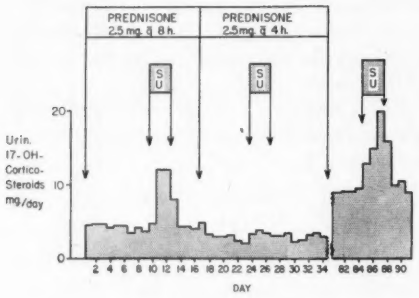

FIG. 3. Responses of a normal man to 0.5 Gm. of SU-4885 every four hours for periods of three days (a) during administration of small doses of prednisone, (b) during administration of larger doses of prednisone, and (c) during a time when no prednisone was being administered.

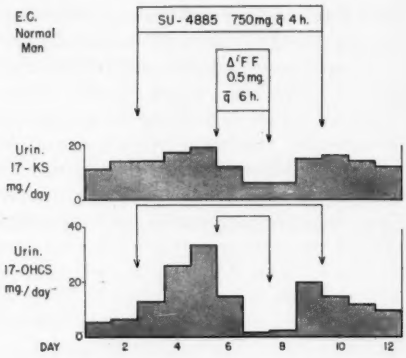

FIG. 4. Response of a normal man to SU-4885 administered alone and in combination with Δ^1-9α-fluorocortisol (Δ^1FF), 0.5 mg. every six hours.

Plasma 17-hydroxycorticoid concentrations (4 subjects) were found to be elevated by the eighth hour of treatment with SU-4885 and were twice the normal values at the end of the 24-hour treatment period. Normal values were observed again twenty-four to forty-eight hours following cessation of treatment.

ACTH was not detectable in the blood prior to treatment (1 subject) but was found to be increased to easily measurable values during treatment with SU-4885.[1]

Normal adults receiving exogenous corticoids or ACTH

Nine subjects were studied during various periods in which SU-4885 was given either concurrently with, or within two days following exogenous corticoid therapy.

Depending upon the dosage of exogenous corticoid, varying degrees of suppression of the normal response to SU-4885 were observed. For example, 15 to 30 mg. of prednisone or 2 mg. of Δ^1-9α-fluorocortisol daily completely suppressed the usual increases in urinary 17-hydroxycorticoids induced by SU-4885. Smaller dosages of the same exogenous corticoids only partially suppressed the response (Fig. 3).

Another type of study illustrating the influence of ACTH-suppressing

[1] Grateful acknowledgement is made to Dr. George Sayers and Dr. Paul Royce of Western Reserve University, Cleveland, Ohio, for performing the assays of blood ACTH content.

corticoids upon the SU-4885 phenomenon is represented in Figure 4. The potent synthetic corticoid Δ^1-9α-fluorocortisol was administered only after SU-4885 had already induced a high output of 17-hydroxycorticoids. During treatment with the ACTH-suppressing steroid, urinary 17-hydroxycorticoids fell to almost negligible levels. Following withdrawal of the ACTH-suppressing steroid, urinary 17-hydroxycorticoids gradually rose to supernormal levels once again, finally returning to normal after SU-4885 was discontinued.

It was frequently observed that following withdrawal of exogenous corticoids normal subjects remained "refractory" to SU-4885 for periods of one to three days. Similar "refractoriness" to SU-4885 was observed following treatment with ACTH. Treatment with repository ACTH preparations (zinc corticotropin) was followed by a sustained increase in plasma and urinary 17-hydroxycorticoids for periods of from twenty-four to forty-eight hours following the last injection. An additional twenty-four to forty-eight hours was required before normal responsiveness to SU-4885 was observed.

An explanation for the "refractoriness" to SU-4885 which occurs with administration of corticoid or ACTH is to be found in the concept that the rise in ACTH secretion which ordinarily occurs during treatment with SU-4885 is actually a *compensatory* response to decreased cortisol levels. As long as levels of cortisol (or its biologic equivalent) are maintained at normal or higher than normal values, then the ACTH-secreting mechanism receives no stimulus to "compensate." Without increased ACTH secretion, there is little if any rise in 17-hydroxycorticoid secretion. An important practical implication of these observations is that exogenous corticoids or ACTH must be avoided when SU-4885 is being used to test for pituitary-adrenal integrity.

Addison's disease

Four studies were performed (Fig. 5). In 2 of the studies the patients received no exogenous steroids except for 0.25 mg. of 2α-methyl-9α-fluorocortisol each day. In dosages of this order, 2α-methyl-9α-fluorocortisol is very effective in promoting sodium retention but has practically no ACTH-suppressing activity (6). Neither of the patients exhibited an appreciable increase in urinary 17-hydroxycorticoids when SU-4885 was administered. This is readily explained by the fact that adrenocortical tissue alone has the capacity to secrete significant quantities of 17-hydroxycorticoids. A normal rise in 17-hydroxycorticoid excretion following treatment with SU-4885 can be used as conclusive evidence in excluding the diagnosis of Addison's disease.

The other 2 studies were performed in a patient who had been subjected

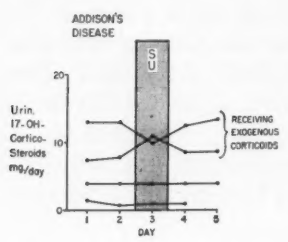

FIG. 5. Lack of response to SU-4885 in 4 patients with Addison's disease.

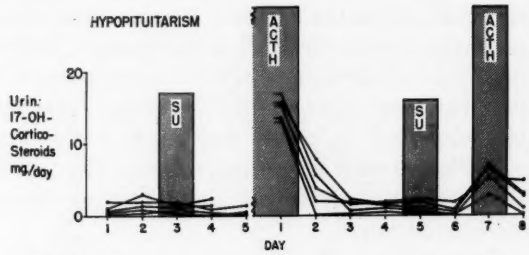

FIG. 6. Lack of response to SU-4885 in 5 patients with frank hypopituitarism. In an effort to restore the capacity of the adrenal glands to respond to endogenous ACTH, repository corticotropin was administered to each patient. When SU-4885 was then administered it still failed to induce an increase in urinary 17-hydroxycorticoids.

to bilateral adrenalectomy and who was being maintained in one study with oral cortisol (40 mg. per day) and in the other study with intramuscular cortisone acetate (50 mg. per day). No consistent change in 17-hydroxycorticoid excretion was observed during or following treatment with SU-4885, indicating that SU-4885 does not cause an increase in the level of plasma or urinary 17-hydroxycorticoids merely as a result of altered extra-adrenal metabolism of steroids.

Hypopituitarism

Five patients were studied. Two had Sheehan's syndrome, 1 had a craniopharyngioma, 1 had idiopathic juvenile panhypopituitarism and 1, a 66-year-old man, had hypopituitarism of unknown etiology. All exhibited clinical and chemical evidence of hypogonadism, hypothyroidism and hypoadrenocorticism. All except the patient with juvenile hypopituitarism were receiving thyroid and gonadal substitution therapy at the time these studies were performed, but none was receiving ACTH or adrenocortical steroids.

SU-4885 failed to induce an appreciable increase in the already subnormal urinary excretion of 17-hydroxycorticoids of any of these patients (Fig. 6).

Conceivably, the failure of these patients to respond to SU-4885 could be explained either by a failure of their damaged pituitary glands to secrete additional ACTH or by inability of the atrophic adrenal glands to respond to ACTH. In order to eliminate the latter explanation, the patients were treated with exogenous ACTH for several days until it was clear that their adrenal glands had recovered the capacity to respond to stimulation. Treat-

ment with ACTH was then discontinued and after three days SU-4885 was administered. In no instance did urinary or plasma 17-hydroxycorticoid levels rise appreciably. After another delay, exogenous ACTH was again administered and evoked in each case a small rise in 17-hydroxycorticoid excretion.

The failure of patients with panhypopituitarism to respond normally to SU-4885, therefore, is explained by the concept that the rise in adrenocortical secretion which normally follows treatment with SU-4885 is mediated by an increase in ACTH secretion by the adenohypophysis. In panhypopituitarism this mediating mechanism is lost. A normal rise in urinary 17-hydroxycorticoids following treatment with SU-4885 can be used as conclusive evidence in excluding the diagnosis of severe panhypopituitarism.

Limited pituitary reserve

There were 16 patients in this category. They shared the following characteristics:

1) All had past histories suggesting hypothalamic or pituitary dysfunction. Six had x-ray evidence of enlargement of the sella turcica, thought in each case to be due to a chromophobe adrenoma. Three had acromegaly and x-ray evidence of enlargement of the sella turcica. Three had been successfully treated for Cushing's disease with cobalt irradiation of the pituitary. One had diabetes insipidus which had developed suddenly following a relatively minor head injury. Two were cachectic as a consequence of anorexia nervosa; one was cachectic as a result of a gastro-ileostomy.

2) All exhibited adequate adrenocortical function for everyday needs, judging by clinical well-being. In the face of infection or trauma, however, 5 patients had shown manifestations of adrenal insufficiency such as vomiting, hyponatremia, hypotension, lethargy and mental confusion, with dramatic improvement following cortisol therapy. Four of the 5 had chromophobe adenomas; the fifth was cachectic as a result of gastro-ileostomy.

3) All exhibited normal or near-normal urinary 17-hydroxycorticoid excretion under nonstressful conditions.

4) All exhibited normal or near-normal increases in urinary 17-hydroxycorticoid excretion during standard intravenous ACTH stimulation tests.

All 16 of these patients failed to respond to SU-4885 with a significant increase in 17-hydroxycorticoid excretion (Fig. 7). This failure to respond to SU-4885 is taken as evidence of impaired pituitary-adrenal reserve. In view of the normal responses to exogenous ACTH it is concluded that the "bottleneck" in this situation must have been at the hypothalamic-pituitary level. Presumably the ACTH-secreting mechanisms of these individuals were already working at rates near their maxima under everyday conditions and had limited capacities to secrete additional ACTH either in re-

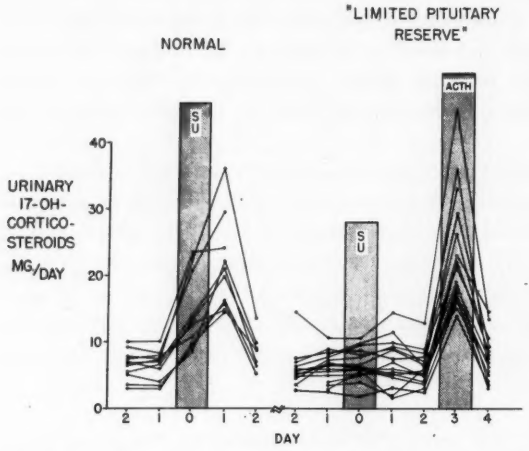

FIG. 7. Responses of normal subjects to SU-4885 compared with the lack of response in patients categorized as having "limited pituitary reserve." Failure of the latter group to exhibit an increase in urinary 17-hydroxycorticoids in response to SU-4885 was not due to inability of the adrenal glands to function, as shown by the fact that they responded well to direct stimulation with exogenous ACTH.

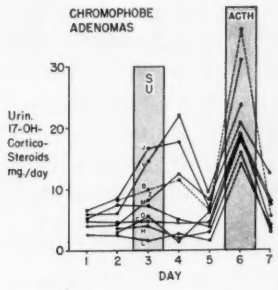

FIG. 8. Responses to SU-4885 in 9 patients with chromophobe adenomas. Six had "limited pituitary reserve" but were able to respond to exogenous ACTH.

sponse to stress or in response to decreased cortisol secretion (the latter a result of treatment with SU-4885).

The observation that patients with "limited pituitary reserve" may, nevertheless, have adrenal glands which are in a normal state of responsiveness implies that those amounts of ACTH which are sufficient to stimulate ordinary adrenocortical secretory activity are also adequate to maintain the adrenal glands in such a state that they are capable of responding vigorously to more intense stimulation. This is reasonable, since it is apparent that normal persons have only "basal" secretion of ACTH under everyday conditions and yet maintain their adrenal glands in a state of responsiveness such that there is an immediate many-fold increase in corticosteroid secretion whenever an increase in endogenous or exogenous ACTH occurs.

Chromophobe adenoma

Nine patients were studied. Important features of each case are summarized in Table 1.

Five patients of this group failed to show an increase in urinary 17-hydroxycorticoids following treatment with SU-4885, though it was subsequently demonstrated that they were capable of responding to exogenous

TABLE 1. ENDOCRINOLOGIC EVALUATION OF 9 PATIENTS WITH CHROMOPHOBE ADENOMAS

	Patient								
	B	C	H	J	L	M	O	Y	Z
Sex	M	M	M	F	F	M	M	F	M
Age (yrs.)	49	27	58	27	60	22	32	50	65
X-ray evidence of sellar erosion	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Previous subtotal resection of adenoma	No	Yes	No	No	Yes	No	Yes	No	No
Previous pituitary irradiation	Yes	No	Yes	No	No	Yes	No	No	No
Urinary gonadotropin (mouse units /24 hrs.)	about 60	under 10	—	under 10	under 10	under 20; over 6	—	over 60	over 5; under 10
Serum protein-bound iodine (μg./100 ml.)	5.0	5.5	5.0	6.0	3.0	4.4	5.2	6.4	2.4
Thyroidal 24-hour I^{131} uptake (%)	—	29	15	—	—	15	15	16	7
Serum cholesterol (mg./100 ml.)	230	255	300	180	300	215	270	340	265
Basal metabolic rate (%)	—	−37	−15	0	—	−25	−25	—	−17
Urinary 17-ketosteroids (mg./24 hrs.)	6	9	2	12	2	9	4	7	3
Previous episodes of adrenal insufficiency during infection or trauma	Yes	No	Yes	No	Yes	No	Yes	No	No
"Limited pituitary reserve" (failure to respond to SU-4885)	Yes	Yes	Yes	No	Yes	Yes	Yes	No	No

ACTH (Fig. 8). One patient (B) exhibited a very slight response, and 3 had normal responses to SU-4885.

The implication of these observations is that chromophobe adenomas frequently, but not invariably, damage the ACTH-secreting mechanisms so that varying degrees of hypoadrenocorticism result. Perhaps in some cases the ACTH-secreting mechanisms are further modified by radiation or surgical treatment of the adenomas.

Acromegaly

Five patients were studied (Fig. 9). All but one (B) showed x-ray evidence of sellar enlargement. Only one (B) had received x-ray therapy to

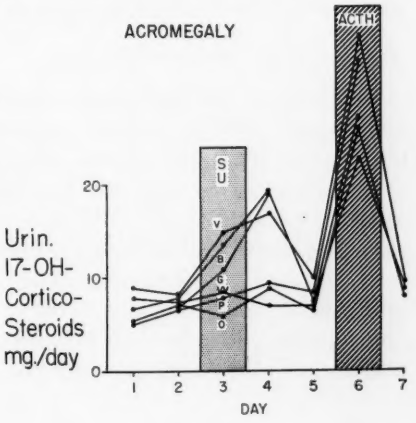

FIG. 9. Responses to SU-4885 in 5 patients with acromegaly. "V" represents a patient prior to pituitary irradiation; "VV" represents the same patient following successful pituitary irradiation. Two patients who had not previously received pituitary irradiation ("O" and "P") also exhibited "limited pituitary reserve."

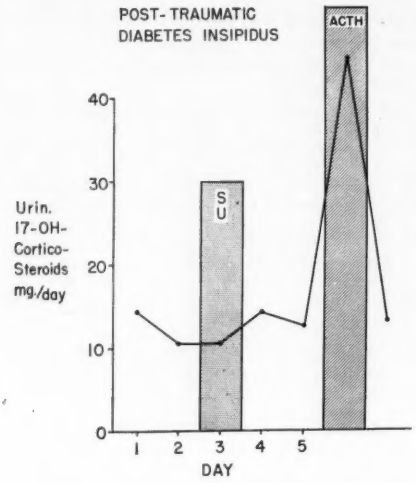

FIG. 10. Failure of a patient with post-traumatic diabetes insipidus to show a normal response to SU-4885 even though adrenocortical responsiveness to ACTH was completely intact.

the pituitary area at some time in the past. All but 2 (O and V) had pituitaries that were judged to be "inactive" with regard to excessive growth hormone secretion at the time of the study. None presented clinical or laboratory evidence of hypopituitarism.

In 3 cases (B, G and V) the responses to SU-4885 were normal. In 2 cases (P and O) the responses to SU-4885 were hardly perceptible even though the responses to standard ACTH tests were normal. In 1 case (V) the response to SU-4885 was normal at the beginning of a course of pituitary irradiation but was negligible at the conclusion of treatment.

These observations imply that some patients with eosinophilic adenomas of the pituitary have limited capacities to secrete ACTH under conditions which normally elicit an increase in secretion of this hormone.

Post-traumatic diabetes insipidus

One patient included in this study was a young man who had been in good health until he suffered a blow to the head, following which severe diabetes insipidus developed. "Basal" 17-hydroxycorticoid excretion was normal, as was the response to exogenous ACTH. However, this patient failed to respond to SU-4885 in a normal fashion (Fig. 10). This is interpreted to mean that the injury which damaged his antidiuretic hormone-secreting mechanism also damaged his ACTH-secreting mechanism so that an SU-4885-induced fall in cortisol secretion failed to bring about a normal compensatory rise in ACTH secretion. This patient exhibited no other abnormalities of pituitary function.

Cachexia

Six patients were studied (Fig. 11). Four were believed to have anorexia nervosa. One had become cachetic following the inadvertent creation of a gastro-ileostomy during a surgical procedure for peptic ulcer. One (O) was a 78-year-old man with "apathetic hyperthyroidism" and anorexia. Except for low urinary gonadotropin values and amenorrhea in 2 patients with anorexia nervosa (B and S), conventional tests of pituitary function revealed no evidence of hypopituitarism in any of these subjects. During exploratory laparotomy the patient with the gastro-ileostomy (T) exhibited manifestations of adrenal insufficiency which responded well to the intravenous administration of cortisol. The remaining 5 subjects were not observed during major episodes of infection or injury.

Two of the patients with anorexia nervosa and the one with the gastro-ileostomy failed to respond to SU-4885. (The SU-4885 was administered intravenously in the latter case.) The other 2 patients with anorexia nervosa and the one with "apathetic hyperthyroidism" responded in normal fashion to SU-4885. All 6 patients responded normally to standard ACTH stimulation tests (Fig. 11).

The results are interpreted as indicating that severe undernutrition may frequently be associated with, and may possibly lead to, impairment of pituitary reserve.

Cushing's syndrome with "adrenal hyperplasia" (diffuse hyperfunction)

Seven patients were studied. Three had been treated previously with pituitary irradiation but had improved only temporarily or not at all. The other patients had received no previous treatment for Cushing's syndrome. All shared the following characteristics:

1) Definite clinical manifestations of chronic excess of cortisol.
2) High urinary excretion of 17-hydroxycorticoids.

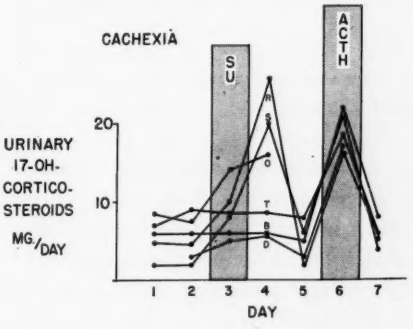

FIG. 11. Responses to SU-4885 in 6 patients with cachexia. Two patients with anorexia nervosa ("B" and "D") and 1 with gastro-ileostomy ("T") exhibited "limited pituitary reserve" although adrenocortical responsiveness was intact.

3) Supernormal responsiveness to standard ACTH stimulation tests.
4) Resistance to suppression of urinary 17-hydroxycorticoids during treatment with small doses (2 mg. per day) of Δ^1-9α-fluorocortisol.
5) Distinct suppressibility of urinary 17-hydroxycorticoids during treatment with very large doses (8 mg. per day) of Δ^1-9α-fluorocortisol.

All of these patients responded to SU-4885 in a qualitatively normal fashion (Fig. 12). The absolute values for urinary 17-hydroxycorticoids following treatment with SU-4885 were higher than those observed in normal subjects tested in the same manner.

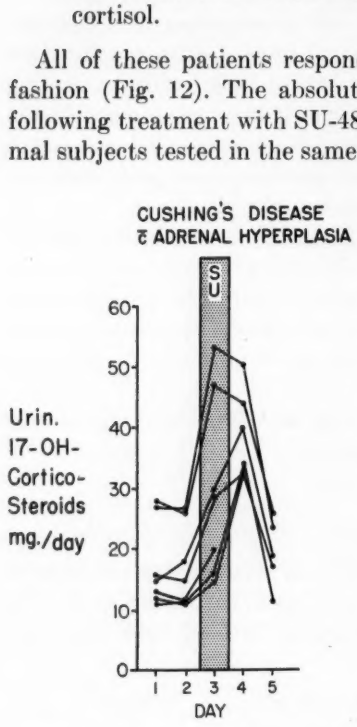

FIG. 12. Responses to SU-4885 in 7 patients with Cushing's disease associated with adrenocortical hyperplasia. In all cases the responses were greater than the average response of normal subjects.

The explanation for the response to SU-4885 in these patients is similar to that invoked in the case of endocrinologically normal subjects: SU-4885 induces a drop in cortisol secretion below the level which is required to restrain ACTH secretion; a compensatory increase in ACTH occurs, inducing in turn an increase in adrenocortical secretion, the most conspicuous product being (under these circumstances) compound S. In patients with Cushing's disease with adrenal hyperplasia, unusually large quantities of cortisol are required to restrain ACTH secretion; impairment of cortisol secretion, therefore, calls forth a vigorous compensatory response by the pituitary which evokes, in turn, an exaggerated secretory response by the adrenal cortices.

Cushing's disease after successful treatment with pituitary irradiation

Four patients were studied. Prior to pituitary irradiation all had exhibited characteristic clinical and laboratory signs of excessive cortisol secretion, resistance to the suppressive action of *small* doses of Δ^1-9α-fluorocortisol, and susceptibility to the suppressive action of *large* doses of Δ^1-9α-fluorocortisol. Following irradiation of the pituitary the clinical manifestations of cortisol excess regressed, and blood and urinary 17-hydroxycorticoid levels returned to normal. The patients still exhibited abnormal resistance to the suppressive action of Δ^1-9α-fluorocortisol; none presented clinical

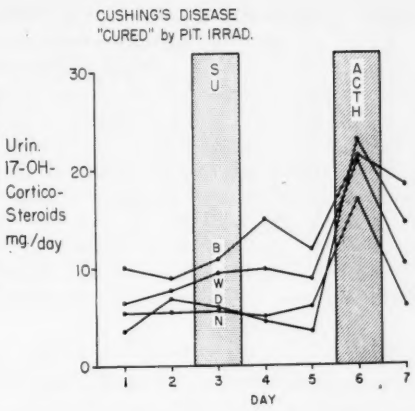

FIG. 13. Responses to SU-4885 in 4 patients who had formerly had Cushing's disease associated with adrenocortical hyperplasia. All had previously been successfully treated by means of pituitary irradiation. As judged by responses to SU-4885, all exhibited evidence of "limited pituitary reserve" with the exception of 1 ("B") who manifested an appreciable, though subnormal, response.

or laboratory evidence of hypothyroidism, hypogonadism or hypoadrenocorticism.

All of these patients showed impaired ability to respond to SU-4885 (Fig. 13). In 3 the responses were negligible; in the fourth the response was appreciable but subnormal. In all cases the adrenal responses to exogenous ACTH were normal.

On the basis of the findings with the SU-4885 test these patients were included in the category of "limited pituitary reserve." It is inferred that successful treatment of Cushing's disease by pituitary irradiation decreases the basal secretion of ACTH, not by restoring the hypothalamico-pituitary system to its normal state (in which it is easily suppressible with steroids, yet vigorously responsive to cortisol withdrawal) but, rather, by crippling the mechanism to the point that while working at its maximum capacity it secretes an amount of ACTH which happens to coincide with everyday requirements.

Cushing's syndrome due to functioning adrenocortical tumor

Three cases were studied.[2] In all 3 there was definite clinical evidence of excessive secretion of cortisol. Plasma and urinary 17-hydroxycorticoid levels were markedly elevated. Responses to standard intravenous ACTH stimulation were negligible in Patients S and Wi but supernormal in Patient We. In no instance was there significant suppression of urinary 17-hydroxycorticoid excretion even with large doses (8 mg. daily) of Δ^1-9α-fluorocortisol. Following removal of adrenal adenomas from 2 of the patients (S and We), basal 17-hydroxycorticoid excretion fell to subnormal

[2] The authors are indebted to Dr. Monte A. Greer, University of Oregon School of Medicine, Portland, Oregon for observations on Patient Wi.

TABLE 2. EFFECT OF SU-4885 UPON URINARY 17-HYDROXYCORTICOSTEROIDS (MG./24 HRS.) IN PATIENTS WITH CUSHING'S SYNDROME ASSOCIATED WITH ADRENOCORTICAL TUMOR

Patient	No treatment				SU-4885		No treatment	
	Day −4	Day −3	Day −2	Day −1	Day 1	Day 2	Day +1	Day +2
S	20	17	18	11	6	5	5	6
We	30	27	24	24	22		20	20
Wi	54	59	45	61	30		29	41

levels and the adrenocortical responsiveness to exogenous ACTH was as poor as that seen in adrenocortical atrophy. The third patient (Wi) proved to have an adrenocortical carcinoma.

Prior to operation none of these patients exhibited increases in urinary 17-hydroxycorticoids in response to SU-4885 (Table 2). In all cases there were marked clinical changes suggestive of "relative" adrenal insufficiency, such as decreased blood pressure, asthenia, anorexia, an increase in circulating eosinophils, decreases in the degree of hyperglycemia, and correction of hypokaliemic alkalosis. Such changes were never prominent in other patients in this study. The failure of patients with Cushing's syndrome due to adrenocortical tumor to exhibit increases in urinary 17-hydroxycorticoid levels following administration of SU-4885 is in marked contrast to the results obtained in patients with diffuse adrenocortical hyper function.

It seems probable that the ACTH-secreting mechanisms of these 3 patients were normally suppressible with cortisol. Unless SU-4885 caused almost complete inhibition of cortisol secretion by the adrenal tumors, it is possible that circulating cortisol might not have fallen to truly subnormal levels and that the ACTH-secreting mechanisms were, consequently, not activated. In the 2 patients (Wi and S) whose tumors were unresponsive to ACTH, it would have been impossible for a normal increase in total 17-hydroxycorticoids to have occurred even if the pituitary itself had responded to SU-4885. Perhaps in Patient We some activation of the pituitary did occur; at least her total 17-hydroxycorticoid excretion did not fall as much as in the other 2 cases.

The fact that total 17-hydroxycorticoid values actually underwent decreases in 2 patients with autonomous adrenal function suggests that SU-4885 in the dosages employed did more than merely inhibit 11β-hydroxylation. Simple inhibition of 11β-hydroxylation might have been expected to lead to equimolar substitution of 11-desoxycortisol for cortisol in the adrenal effluent. Although inhibition of 11β-hydroxylation has been shown

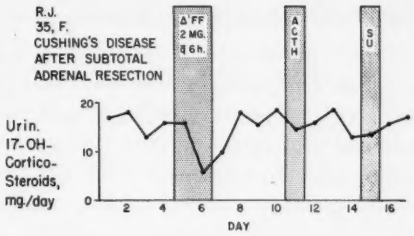

FIG. 14. Studies of a woman with Cushing's disease associated with adrenocortical hyperplasia, who had previously undergone subtotal adrenalectomy. Basal 17-hydroxycorticoid excretion was elevated. Response to exogenous ACTH was negligible. Endogenous ACTH was elevated. Endogenous adrenal secretion could be suppressed only with very large dosages of exogenous corticoids. Total 17-hydroxycorticoid excretion was not altered by SU-4885 therapy. (The hyperadrenocorticism of this patient was not modified by pituitary irradiation but was ultimately corrected by hypophysectomy.)

to be the most prominent effect of SU-4885 in low concentrations, it appears that some degree of inhibition of still other steps in steroid biosynthesis occurred with the dosages employed in this study.

Limited adrenocortical reserve

One patient (R.J.) was studied in whom subtotal adrenalectomy had been performed two years previously as treatment for Cushing's syndrome. During the two-year interval there had been a recurrence of the clinical syndrome, and urinary 17-hydroxycorticoids (which had averaged 28 mg. per day preoperatively and 8 mg. per day postoperatively) were found to have risen again to approximately 15 mg. per day. The fact that 17-hydroxycorticoid secretion could be suppressed with large doses of Δ^1-9α-fluorocortisol indicated that the adrenal hypersecretion was ACTH-dependent. The fact that no increase in 17-hydroxycorticoid levels occurred in response to a standard intravenous ACTH stimulation test indicated that the adrenal remnant was already responding maximally to high levels of endogenous ACTH. (ACTH was found in measurable—and therefore supernormal—concentrations in this patient's blood by Dr. George Sayers and Dr. Paul Royce.)

In response to SU-4885 this patient exhibited no rise in total urinary 17-hydroxycorticoids (Fig. 14).

This failure to respond in normal fashion to SU-4885 is interpreted as indicating that the adrenal remnant was already working at its maximum rate and had no reserve capacity to respond to further increases in endogenous ACTH. On the other hand, since the increase in endogenous ACTH was not actually measured, it is also conceivable that this patient's pituitary was already secreting ACTH at its maximum rate and had no further reserve capacity with which to respond to an SU-4885-induced fall in the secretion of cortisol. The two possible explanations are not mutually exclusive.

Congenital adrenal hyperplasia

Four patients with virilizing congenital adrenal hyperplasia were studied. One, a 9-year-old boy, had hypertension and moderately elevated urinary 17-hydroxycorticoids and 17-ketosteroids. Tetrahydro-11-desoxycortisol and tetrahydro-11-desoxycorticosterone were both present in his urine prior to treatment with SU-4885. The other 3 subjects had normal blood pressure, normal basal 17-hydroxycorticoids and markedly elevated 17-ketosteroids. None had a history of salt wasting.

All of the patients in this group responded to SU-4885 with increases in urinary 17-hydroxycorticoids. Except in the boy with hypertension, the responses were subnormal (Fig. 15).

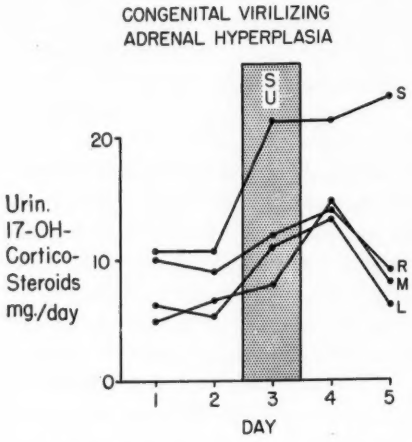

FIG. 15. Responses to SU-4885 in 4 patients with virilizing congenital adrenal hyperplasia. The excretion of 17-hydroxycorticoids was highest in Patient "S," a 9-year-old boy with hypertension. Although a definite response to SU-4885 occurred in every case, the magnitude of the response in the nonhypertensive patients was less than that in normal subjects.

This is interpreted as meaning that even though the adrenocortical enzyme systems for biosynthesis of cortisol were inefficient, requiring a compensatory increase in ACTH secretion, these patients nevertheless had some pituitary-adrenal "reserve." Thus when their cortisol-secreting mechanisms were further impaired by SU-4885 they were capable of a further increase in secretion of ACTH with a resultant increase in secretion of 11-desoxycortisol.

The response of the boy with the hypertensive form of congenital adrenal hyperplasia was especially interesting. SU-4885 itself, by inhibiting 11β-hydroxylation, induces changes in adrenal steroid patterns of normal subjects which simulate those described by Eberlein and Bongiovanni in a patient with the hypertensive form of congenital adrenal hyperplasia (7). Like the patient of Eberlein and Bongiovanni, the hypertensive patient in the present study excreted metabolites of 11-desoxycortisol and 11-desoxycorticosterone. This may be taken as evidence that the congenital defect

did not result in complete loss of 11β-hydroxylating capacity. In fact, in detailed analysis of the steroid content of this patient's urine prior to treatment with SU-4885, tetrahydrocortisol plus tetrahydrocortisone accounted for approximately 20 per cent of the total urinary Porter-Silber chromogens.

Primary hypothyroidism with myxedema

Six patients were studied. In every case myxedema was clinically obvious, and serum protein-bound iodine concentration, thyroidal radioiodine uptake and basal metabolic rate were markedly depressed. Although "basal" values for urinary 17-hydroxycorticoids were subnormal in 3 instances adrenocortical responsiveness to exogenous ACTH approached normal in all 6 subjects.

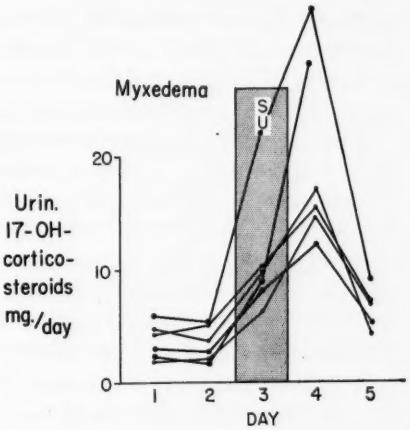

FIG. 16. Responses to SU-4885 in 6 patients with frank myxedema due to primary hypothyroidism. Although basal 17-hydroxycorticoid excretion levels were subnormal, pituitary reserve was unimpaired.

In all cases a normal rise in urinary 17-hydroxycorticoids occurred following treatment with SU-4885 (Fig. 16).

This is interpreted to mean that loss of pituitary responsiveness with respect to ACTH secretion is not characteristic of hypothyroidism of moderate severity.

DISCUSSION

It is proposed that in SU-4885 we have a means of testing the reserve capacity of the pituitary-adrenal system to satisfy extraordinary physiologic needs for cortisol. Heretofore it has been possible by means of clinical evaluation, 17-hydroxycorticoid assays, and the standard ACTH stimulation test to assess the adequacy of adrenal function under "basal" conditions, but there has been no convenient way to determine whether the pituitary gland possessed reserve capacity to secrete additional ACTH. This information may now be made available through employment of the SU-4885

test. Ideally, one would like to measure ACTH itself in studies designed to test pituitary secretion of that hormone. The available methods are not sufficiently sensitive to measure circulating ACTH under most physiologic conditions. In testing pituitary activity with SU-4885 the assumption is made that, provided the mechanism for adrenal responsiveness is intact, major changes in ACTH secretion will be reflected in parallel changes in blood or urinary corticoid levels.

The results obtained using the SU-4885 test in a variety of clinical situations appear to substantiate the validity of the test. Under circumstances in which inhibition of cortisol secretion would not be expected to result in a compensatory increase in ACTH secretion, no response to SU-4885 occurred. Examples of such situations included panhypopituitarism and "hypopituitarism-with-respect-to-ACTH" induced in normal subjects by administration of corticoids. Normal subjects, on the other hand, regularly responded to SU-4885 inhibition of cortisol secretion with an increase in urinary 17-hydroxycorticoid excretion, which was observed to be associated with increased levels of circulating ACTH.

It was of interest that certain patients with pituitary lesions but without frank hypopituitarism could be demonstrated by means of the SU-4885 test to have limited capacity to secrete more than "basal" quantities of ACTH. Despite the fact that several patients with "limited pituitary reserve" (as judged by poor responses to the SU-4885 test) had previously experienced clinical adrenal insufficiency in the face of trauma or infection, it is not yet clear whether failure of an individual to respond normally to SU-4885 necessarily implies inability to respond to injury with an increase in ACTH secretion. It is conceivable that inhibition of cortisol secretion by means of SU-4885 might provide a relatively mild stimulus to ACTH secretion and that a stress such as a major surgical procedure might provide a more intense stimulus. If so, then patients with minor degrees of hypothalamico-hypophyseal insufficiency might fail to respond to SU-4885 while retaining the capacity to respond to injury. We have observed thus far 2 patients who had "limited pituitary reserve" as judged by their lack of response to the standard SU-4885 test but who were, nevertheless, able to survive extensive surgical procedures without the support of exogenous steroids. In one of these patients a significant increase in plasma and urinary 17-hydroxycorticoid levels was observed during a pulmonary resection. The SU-4885 test, therefore, appears to be more sensitive than any measure heretofore available for the detection of defects in ACTH-secreting efficiency. How reliable the test might be in predicting the pituitary-adrenal response to injury remains to be seen.

Application of the SU-4885 test to patients with Cushing's syndrome has added to our information concerning the basic nature of that disorder.

The fact that patients with adrenal "hyperplasia" responded vigorously to SU-4885 whereas those with adrenal tumors did not, is consistent with the view that the former are only quantitatively different from normal whereas the latter are qualitatively different from normal with respect to homeostatic regulation of adrenal function.

The application of the SU-4885 test deserves consideration in still other situations in which information is needed concerning pituitary reserve. An obvious example is that of determining whether pituitary-adrenal hypofunction which follows prolonged treatment with corticoids is due solely to adrenocortical atrophy or whether there is also a loss of pituitary responsiveness to needs for corticoids.

In the judgment of the authors, caution should be exercised in the quantitative interpretation of the SU-4885 test. The purpose of the present study has been to explore the question of whether in patients with pituitary or adrenal disorders there exist meaningful deviations from normal in the response of the pituitary-adrenal system to a decrease in cortisol secretion. The results presented indicate that such deviations do exist. As carried out in this study, the SU-4885 test provides only a gross index of pituitary reserve, and it would seem inadvisable to attempt a precise quantitative interpretation of the results of the test at this time. A strict quantitative interpretation of the pituitary response to SU-4885 should take into consideration the rate and degree of fall in the level of circulating cortisol, the rate of recovery of cortisol-secreting efficiency after SU-4885 has been withdrawn, the responsiveness of the adrenal to graded dosages of ACTH, the rate of metabolism and excretion of compound S and its metabolites, the proportion of secreted compound S which is excreted as "tetrahydrocompound-S-glucuronide" in a given subject, the relative proportions of the various steroids which make up the total 17-hydroxycorticoids, and the "chromogenicity" of the various steroids measured by the Porter-Silber reaction (this factor alone varies from one laboratory to another depending upon the conditions employed in color development).

For the purpose of the present study we have considered that any unequivocal increase in the urinary excretion of total 17-hydroxycorticoids following treatment with SU-4885 is an indication that the subject must possess some measure of "pituitary reserve." Responses which were of lesser degree than the least response seen in our series of 11 normal subjects (Fig. 2) have been regarded as subnormal. The dosage of SU-4885 was of some importance. Ancillary observations indicated that in any one subject the response to 750 mg. of SU-4885 every four hours was slightly greater than the response to 500 mg. These dose-response differences were small, however, compared with the differences in responses which distinguished normal subjects from those with "limited pituitary reserve."

Acknowledgments

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ADRENOCORTICAL RESPONSE TO THE 48-HOUR ACTH TEST IN MYXEDEMA AND HYPERTHYROIDISM*

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ABSTRACT

The response of urinary 17-hydroxycorticosteroids (17-OH-CS) and 17-ketosteroids (17-KS) to the standard 48-hour intravenous ACTH test was investigated in 12 patients with hyperthyroidism and in 9 with myxedema. Control values for urinary 17-OH-CS were low in myxedema and slightly elevated in hyperthyroidism. Control values for urinary 17-KS were low in both myxedema and hyperthyroidism. In hyperthyroidism, there was a normal response of urinary 17-OH-CS excretion on the first day of ACTH stimulation but no further increase on the second day. This indicates some degree of impairment of adrenocortical reserve. In myxedema, the patients could be classified into 2 categories. One group exhibited a subnormal urinary steroid response on both days of ACTH stimulation, whereas the other group showed a normal response on the first day but a supra-normal response on the second day; moreover the elevated urinary 17-OH-CS persisted on the day following cessation of ACTH. It is suggested that in hyperthyroidism the production of adrenocortical steroids is increased, although there may be a decreased reserve. In myxedema, the production of adrenocortical steroids appears to be reduced but the response to ACTH stimulation varies from below normal to above normal.

THE effects of the level of thyroid activity on the functional status of the adrenal cortex has been the subject of investigation in several laboratories. Different groups of investigators have reported that plasma levels of free corticoids in myxedema and hyperthyroidism are within the

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usually accepted range of normal (1–7). In patients with classic myxedema, the urinary levels of 17-hydroxycorticoids (17-OH-CS) and 17-ketosteroids (17-KS) are decreased (1, 2, 4, 5, 8, 9). On the other hand, in hyperthyroidism the urinary 17-OH-CS excretion is increased whereas the 17-KS excretion is in the low range of normal (1, 2, 4, 5, 9). The rate of disappearance of hydrocortisone from plasma has been shown to be modified by the status of thyroid function, being prolonged in patients with myxedema and shortened in those with hyperthyroidism (1–4, 10, 11).

Thus, results of previous investigations indicate that in myxedema there is decreased production, a decreased rate of disappearance from plasma and decreased urinary excretion of 17-OH-CS, whereas in hyperthyroidism there is increased production, an increased rate of disappearance from plasma and slightly increased excretion of 17-OH-CS. In both extremes of thyroid activity, plasma homeostasis of 17-OH-CS is maintained.

In myxedema the adrenocortical responsiveness to stimulation with adrenocorticotropin (ACTH), as measured by increased levels of urinary 17-KS, has been reported to be subnormal (12). The urinary 17-OH-CS response to a single 8-hour intravenous infusion of ACTH was found to be normal in both myxedema and hyperthyroidism (5). In thyrotoxicosis the urinary 17-OH-CS response to an 8-hour intravenous infusion of ACTH repeated for three consecutive days was shown to decrease gradually from the first to the third day (6, 7). The site and mode of action of the thyroid hormones on adrenocortical function remain obscure. The present study was undertaken to determine the adrenocortical response to the 48-hour intravenous ACTH test in patients with myxedema and hyperthyroidism in an effort to elucidate further the metabolic interrelationships which exist between the thyroid and the adrenal glands.

METHODS AND MATERIALS

Twenty-one female patients were studied on the metabolic ward of the Peter Bent Brigham Hospital—9 with myxedema and 12 with hyperthyroidism. Ten normal women served as controls.

In evaluating adrenocortical function in each patient, two 24-hour urine collections were obtained to determine the baseline excretion of 17-OH-CS and 17-KS. Following this, the response to an 8-hour intravenous infusion of ACTH[1] (25 U.S.P. units in 500 ml. of physiologic sodium chloride) was determined on two consecutive days, as previously reported from this laboratory (13–15).

Total urinary 17-OH-CS were determined by a modification of the Reddy-Jenkins-Thorn method for the estimation of 17-OH-CS in urine (16). Urinary 17-KS were measured by a modification of the method of Drekter and associates (17). Urine collections were checked by creatinine determinations, using the technique of Bonsnes and Taussky (18). Only those patients who were judged to have accurate urine collections on the basis of the urinary creatinine values were included in the study.

[1] Kindly supplied by the Upjohn Company, Kalamazoo, Michigan.

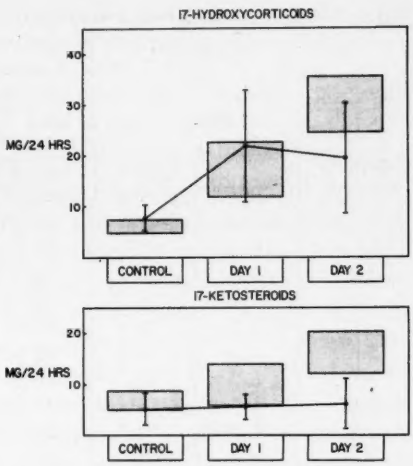

FIG. 1. Urinary 17-OH-CS and 17-KS values before and during the 48-hour intravenous ACTH test in hyperthyroidism. The shaded areas represent the upper and lower limits of the standard deviation from the mean in normal subjects. The vertical lines represent plus or minus 1 standard deviation about the mean. The means are connected by a solid line.

Thyroidal 24-hour uptakes of radioactive iodine (after a dose of 10 to 15 microcuries of I^{131}) were determined by means of a scintillation counter. Serum protein-bound iodine levels were determined at the Boston Medical Laboratory by the method of Zak and associates (19).

RESULTS

Hyperthyroidism

In the 12 subjects with hyperthyroidism (Table 2, Fig. 1) the diagnosis was established by thorough clinical evaluation and confirmed by serum protein-bound iodine and cholesterol levels and the 24-hour thyroidal uptake of radioactive iodine. Control values for urinary 17-OH-CS were in the upper part of the normal range; the mean value was higher than normal. The urinary 17-OH-CS response to stimulation with ACTH varied within a wide range. In contrast to the responses obtained in normal subjects (Table 1), no further rise occurred on the second day of ACTH stimulation, and in some instances the increments over the control values were lower than those observed on the first day. The D2/D1 ratio (which represents the increment above control values of the second day divided by that of the first day of ACTH stimulation) was lower than the ratio in the normal control subjects, indicating no potentiation by the first day of ACTH stimulation. This ratio was calculated in order to compare the response on the second day of ACTH stimulation to the response on the first day of stimulation. It represents a measure of the potentiating effect that the first day of ACTH administration has on the stimulation produced on the second day. There did not appear to be any correlation between the adrenocortical response and the functional status of the thyroid. Control values

TABLE 1. URINARY 17-HYDROXYCORTICOIDS AND 17-KETOSTEROIDS BEFORE AND DURING THE 48-HOUR INTRAVENOUS ACTH TEST IN 10 NORMAL WOMEN AGED 20–35 YEARS

Subject	17-OH-CS (mg./24 hrs.)			D2/D1 Ratio†	17-KS (mg./24 hrs.)		
	Control	Day 1 ACTH*	Day 2 ACTH*		Control	Day 1 ACTH*	Day 2 ACTH*
M.A.	6.3	11.3	25.6	2.26	5.2	12.8	18.0
A.B.	6.0	25.7	29.2	1.13	5.3	8.2	17.1
M.H.	5.7	10.3	24.4	2.36	7.4	5.9	9.2
S.L.	5.6	17.1	30.7	1.79	4.5	6.6	11.4
J.L.	8.9	18.2	23.8	1.30	9.5	11.9	20.7
N.P.	5.2	21.8	39.7	1.82	8.1	18.4	20.8
B.R.	6.7	13.6	34.7	2.55	9.0	8.2	18.2
J.R.	4.3	20.6	31.0	1.50	5.8	7.3	14.6
A.E.	1.4	23.3	37.4	1.65	13.4	16.6	25.8
V.V.	3.7	19.4	21.2	1.09	7.8	9.3	9.6
Mean	5.4	18.1	29.8	1.75	7.6	10.5	16.5
±S.D.	1.5	5.1	6.5	0.25	2.7	4.3	5.4

* Increment above control values.

† The ratio of the increment (above control values) on the second day to that on the first day.

TABLE 2. PARAMETERS OF THYROID FUNCTION AND URINARY 17-HYDROXYCORTICOIDS AND 17-KETOSTEROIDS BEFORE AND DURING THE ACTH TEST IN 12 PATIENTS WITH HYPERTHYROIDISM

Patient		Serum PBI (μg./100 ml.)	Thyroidal 24-hr. I^{131} uptake (% dose)	Serum chol. (mg./100 ml.)	17-OH-CS excretion (mg./24 hrs.)			D2/D1 ratio†	17-KS excretion (mg./24 hrs.)		
Name	Age (yrs.)				Control	Day 1 ACTH*	Day 2 ACTH*		Control	Day 1 ACTH*	Day 2 ACTH*
M.T.	45	10.8	78	142	5.7	9.2	12.2	1.3	4.9	8.9	0.0
E.N.	54	11.4	86	176	6.6	12.4	14.9	1.2	3.4	3.7	5.0
I.M.	40	10.8	78	185	7.9	14.5	8.8	0.6	3.5	3.8	4.5
J.T.	51	—	91	106	6.9	14.6	13.1	0.9	2.0	2.0	2.5
A.H.	41	10.0	80	157	9.5	15.2	13.0	0.85	5.3	2.5	1.6
E.P.	58	13.6	80	173	8.4	17.0	15.8	0.9	3.8	3.6	6.1
A.P.	63	12.4	75	206	7.5	22.3	9.6	0.4	5.5	6.5	15.7
R.K.	52	19.3	92	194	6.9	22.9	21.6	0.9	4.6	4.3	10.4
C.C.	62	12.0	72	106	2.7	24.6	15.8	0.6	3.1	8.8	2.1
E.H.	51	14.2	66	105	11.7	28.8	33.3	1.15	7.2	6.6	4.0
C.S.	49	13.2	84	200	10.7	33.6	34.6	1.0	14.4	7.6	12.2
A.C.	46	20.0	71	163	11.4	48.3	41.6	0.9	4.6	4.3	10.4
Mean		13.4	79.5		7.8	21.9	19.5	0.9	5.2	5.6	6.2
±S.D.		3.3	8.2		2.5	10.9	10.9	0.25	3.2	2.5	4.8

* Increment above control values.

† The ratio of the increment (above control values) on the second day to that on the first day.

for urinary 17-KS were, with 2 exceptions, below the mean value found in normal subjects. The 17-KS response to ACTH was definitely below normal.

Myxedema

Nine subjects with classic myxedema were studied (Table 3). The diagnosis was established by clinical evaluation, by measurement of the thyroidal 24-hour uptake of radioactive iodine and by serum levels of protein-bound iodine and cholesterol. Control values for urinary 17-OH-CS were in the low range of normal in all cases; the mean value was lower than normal. Following stimulation with ACTH 4 subjects (Group A) excreted subnormal amounts of 17-OH-CS on both days, and 5 subjects (Group B) excreted amounts that were normal on the first day and above normal on the second day. The difference in response between these two groups is graphically represented in Figure 2. In both groups the 17-OH-CS excretion on the second day was much higher than on the first day. This is expressed by a higher than normal D2/D1 ratio. The control 17-KS values and the response to ACTH were below normal in both groups when compared to the

TABLE 3. PARAMETERS OF THYROID FUNCTION AND URINARY 17-HYDROXYCORTICOIDS AND 17-KETOSTEROIDS BEFORE AND DURING THE ACTH TEST IN PATIENTS WITH MYXEDEMA

Patient		Serum PBI (μg./100 ml.)	Thyroidal 24-hr. I^{131} uptake (% dose)	Serum chol. (mg./100 ml.)	Urinary FSH (m.u.)	17-OH-CS excretion (mg./24 hrs.)			D2/D1 ratio†	17-KS excretion (mg./24 hrs.)		
Name	Age (yrs.)					Control	Day 1 ACTH*	Day 2 ACTH*		Control	Day 1 ACTH*	Day 2 ACTH*
Group A												
S.D.	86	1.6	5.0	360	—	4.2	5.9	13.5	2.2	4.2	1.1	4.3
M.S.	58	0.6	5.0	550	+200	5.3	4.5	13.0	2.0	2.1	0.6	3.3
A.P.	45	1.6	0	471	—	4.7	5.3	19.7	3.7	2.3	2.2	4.5
E.D.	69	1.4	13.0	440	+ 10	4.4	10.0	18.1	1.8	4.5	0.0	5.7
Mean		1.3	5.8			4.7	6.4	16.1	2.4	3.3	1.0	4.4
±S.D.		0.5	5.3			0.8	2.4	3.3	0.8	1.2	0.9	1.0
Group B												
A.H.	62	1.6	0	490	—	2.9	14.1	49.1	3.5	5.4	0.1	9.3
H.F.	54	1.2	18.0	330	—	2.7	18.6	41.6	2.2	2.4	4.8	9.6
I.E.	58	1.2	16.0	390	+100	3.7	19.0	46.9	2.4	3.1	—	13.1
M.H.	56	0.8	0	405	—	4.8	20.2	36.7	1.8	5.3	5.2	13.3
E.Z.	49	1.4	7.0	326	+ 20	3.7	23.7	59.7	2.5	5.2	11.7	29.8
Mean		1.2	8.2			3.6	19.1	46.8	2.5	4.3	5.4	15.0
±S.D.		0.9	7.5			1.0	3.4	8.6	0.6	1.4	4.4	8.5
Groups A and B												
Mean		1.3	7.1			4.1	13.5	33.1	2.45	3.8	3.2	10.3
±S.D.		1.1	7.0			0.87	—	—	0.7	1.4	—	—

* Increment above control values.

† The ratio of the increment (above control values) on the second day to that on the first day.

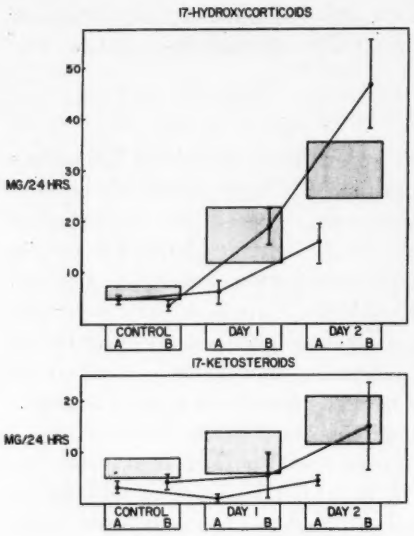

FIG. 2. Urinary 17-OH-CS and 17-KS values before and during the 48-hour intravenous ACTH test in myxedema. The shaded areas represent the upper and lower limits of the standard deviation from the mean in normal subjects. The vertical lines represent plus or minus 1 standard deviation about the mean. The means are connected by a solid line.

values obtained in normal subjects. There did not appear to be any correlation between adrenocortical response and the levels of serum protein-bound iodine, cholesterol, thyroidal radioactive iodine uptake, clinical status of the patient, or known duration of illness.

Post-ACTH urinary 17-OH-CS excretion

The levels of urinary 17-OH-CS on the day following cessation of the two-day ACTH stimulation test are shown in Table 4. In normal subjects on the day following cessation of ACTH, urinary 17-OH-CS returned to control levels or slightly below. This also occurred in the 4 patients with hyperthyroidism and in half (Group A) of the 6 patients with myxedema who were studied. In contrast, the other half (Group B) of the patients with myxedema who were similarly studied exhibited persistence of the elevation of urinary 17-OH-CS levels on the day following cessation of ACTH stimulation. This persistence was not due to a decreased 24-hour clearance of corticoid metabolites, as indicated by the fact that following administration of hydrocortisone to subjects with either hyperthyroidism or hypothyroidism the urinary steroidal increment occurred well within the 24-hour period of collection; moreover, there was no significant carry-over on the following day, as shown by the return to control values. Thus, one might expect that if the urine were collected in serial two-hour to four-hour periods following administration of hydrocortisone, alterations in plasma

TABLE 4. URINARY 17-HYDROXYCORTICOID VALUES ON THE DAYS OF, AND THE DAY FOLLOWING THE 48-HOUR ACTH TEST

Patient	Control (mg./24 hrs.)	Increment or decrement* (mg./24 hrs.)		
		Day 1 ACTH	Day 2 ACTH	Day 3 Post-ACTH
Hyperthyroidism				
J.T.	6.9	+14.6	+13.1	+ 2.0
E.P.	8.4	+17.0	+15.8	− 3.0
A.P.	7.5	+22.3	+ 9.6	− 1.7
A.C.	11.4	+48.3	+41.6	−10.0
Myxedema (Group A)				
S.D.	4.2	+ 5.9	+13.5	+ 1.3
M.S.	5.3	+ 4.5	+13.0	− 0.7
A.P.	4.7	+ 5.3	+19.7	− 1.1
Myxedema (Group B)				
I.E.	3.7	+19.0	+46.9	+38.1
M.H.	4.8	+20.2	+36.7	+22.7
E.Z.	3.7	+23.7	+59.7	+43.5

* Increment or decrement in urinary 17-OH-CS values above (+) or below (−) control values.

corticoid clearance might be reflected in the urinary clearance. However, a consideration of the time changes in plasma half-life (from values of 80 to 120 minutes in normal subjects to values of 160 to 200 minutes in myxedema) would not lead one to expect any reflection of this alteration in a 24-hour collection of urine.

Effect of treatment for thyroid disease

Eight of the patients in this study were retested following treatment for thyroid disease. Euthyroidism was confirmed by clinical evaluation and by measurements of the serum protein-bound iodine and cholesterol levels. All patients were judged euthyroid except E.D., who remained hypothyroid as a result of inadequate thyroid substitution therapy. The values for urinary 17-OH-CS and 17-KS before and after treatment are shown in Table 5 and graphically presented in Figure 3. Following thyroid-hormone therapy in the 6 patients with myxedema, the urinary steroid responses to ACTH (whether originally slight or supra-normal) returned to the normal range. In the 2 patients with hyperthyroidism studied following institution of euthyroidism, the responses to ACTH stimulation likewise returned to normal values.

TABLE 5. EFFECT OF ACTH STIMULATION ON URINARY 17-HYDROXYCORTICOIDS AND 17-KETOSTEROIDS BEFORE AND AFTER THERAPY FOR MYXEDEMA AND HYPERTHYROIDISM

Patient	Time re therapy for thyroid disease	Serum PBI (μg./100 ml.)	Serum chol. (mg./100 ml.)	17-OH-CS excretion (mg./24 hrs.)			17-KS excretion (mg./24 hrs.)		
				Control	Day 1 ACTH*	Day 2 ACTH*	Control	Day 1 ACTH*	Day 2 ACTH*
Hyperthyroidism									
E.H.	Before	14.2	105	11.7	28.8	33.3	7.2	6.6	4.0
	After	7.0	212	7.1	44.4	39.4	8.7	21.2	26.3
C.S.	Before	13.2	200	10.4	33.6	34.6	14.4	8.6	12.2
	After	6.6	224	5.4	21.2	27.0	11.9	8.0	12.5
Myxedema (Group A)									
M.S.	Before	0.6	550	5.3	4.5	13.0	3.1	0.6	3.3
	After	2.4	265	5.2	7.6	15.5	4.4	3.2	7.0
A.P.	Before	1.6	471	4.7	5.3	19.7	2.3	2.2	4.5
	After	4.0	273	8.3	20.3	22.6	4.9	7.1	9.3
E.D.†	Before	1.4	440	4.4	10.0	18.1	4.5	0.0	5.7
	After	2.0	440	2.6	15.3	29.0	3.1	6.9	7.0
Myxedema (Group B)									
I.E.	Before	1.2	390	3.7	19.0	46.9	3.1	—	13.1
	After	4.8	234	4.3	15.8	38.5	3.4	6.1	10.6
E.Z.	Before	1.4	326	3.7	23.7	59.7	5.2	11.7	29.8
	After‡	0.2	208	5.2	3.7	20.7	3.6	8.8	25.8
M.H.	Before	0.8	405	4.8	20.2	36.7	5.3	5.2	13.3
	After	—	230	8.7	19.1	24.9	5.5	6.0	5.7

* Increment above control values.
† This patient remained hypothyroid because of inadequate therapy.
‡ This patient was receiving *l*-triiodothyronine, which does not elevate the serum level of protein-bound iodine.

DISCUSSION

The studies of numerous investigators have clearly demonstrated the slightly elevated baseline levels of urinary 17-OH-CS in patients with hyperthyroidism. The results of the present study confirmed this finding. Further, the response to the 48-hour intravenous ACTH test was found to be within normal limits on the *first* day of the test with no further increase on the second day. These observations have been confirmed (6) and extended to show further diminution on the third day of ACTH stimulation. The diminished response on the second day of ACTH administration indicates an inability of the adrenal gland in hyperthyroidism to either maintain or increase production during continued stimulation by ACTH. This inability to maintain an increased rate of secretion may reflect 1) diminished adrenocortical reserve, 2) enhanced destruction of ACTH, 3) enhanced steroid catabolism, or 4) any combination of these. Diminished adrenocortical reserve would appear to be the most reasonable explanation. A similar pattern of response to the 48-hour ACTH test has also been

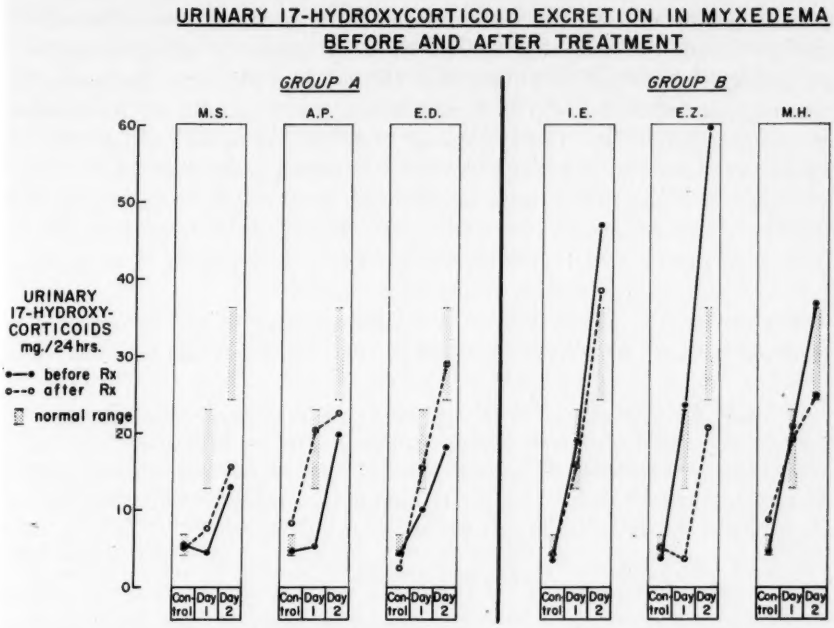

FIG. 3. Effect of thyroid-hormone therapy on the urinary 17-OH-CS response to intravenous ACTH in 6 patients with myxedema. The shaded areas represent the upper and lower limits of the standard deviation from the mean in normal subjects.

found in patients with "partial" adrenocortical insufficiency (2) and is in contrast to the progressive increase in adrenocortical response seen in patients with panhypopituitarism. The low basal urinary 17-KS excretion and the poor response to ACTH stimulation also support this concept of an abnormality in steroidogenesis by the adrenal gland. It is remarkable that there persists such excellent adrenocortical function in patients with severe hyperthyroidism despite the marked increase in production and extremely rapid degradation of adrenal steroids. Indeed, it remains undemonstrated that adrenocortical insufficiency exists in so-called "thyroid crisis," despite the fact that a "relative" insufficiency may occur.

There seems to be little doubt that adrenocortical activity is increased in hyperthyroidism. This concept is supported by the fact that increased levels of urinary corticoids are found in this disorder. The percentage excretion of administered cortisone in patients with hyperthyroidism has been reported as normal (5). It remains to be demonstrated whether or not this increased adrenocortical function is directly due to the generalized increase in metabolism of all tissues in patients with hyperthyroidism or whether it results from increased secretion of pituitary ACTH.

In hyperthyroidism, the dissociation of the elevated control levels of urinary 17-OH-CS from the reduced levels of urinary 17-KS is of particular interest. One may speculate that the adrenal gland is producing increased quantities of 17-OH-CS compounds preferentially at the expense of adrenal 17-KS. The 11-oxygenated 17-KS excretion in normal females has been reported to be of the order of 1.0 to 3.0 mg. per twenty-four hours. Therefore an impairment in the degradation of 17-OH-CS to 11-oxy-17-KS would not satisfactorily explain the low 17-KS values in hyperthyroidism. Abnormal patterns of 17-KS excretion have been reported in myxedema (21) and may similarly exist in hyperthyroidism.

The findings in patients with myxedema appear to be the opposite of those in patients with hyperthyroidism. The results of the present study

TABLE 6. RECOVERY OF 17-HYDROXYCORTICOIDS FROM URINE IN MYXEDEMA AFTER ADMINISTRATION OF HYDROCORTISONE OR CORTISONE

Patient	Diagnosis	Steroid administered	Urinary 17-OH-CS		
			Baseline (mg./24 hrs.)	Recovered after admin. of cortisone	
				(mg./24 hrs.)	(%)
E.Z.	Myxedema	Hydrocortisone, 100 mg.	3.7	15.8	12.1
E.Z.	Myxedema	Hydrocortisone, 200 mg.	2.6	30.6	14.0
L.B.	Myxedema	Cortisone, 100 mg.	2.6	21.7	19.

confirm those of other investigators that there are reduced levels of urinary 17-OH-CS in patients with myxedema. The percentage excretion of administered cortisone in myxedema is normal, as shown in 2 of our subjects (Table 6). It has been reported that an increased percentage of hydrocortisone is recovered as hydrocortisone ("free") in the urine of patients with myxedema (1). This finding probably reflects a decreased rate of degradation of hydrocortisone, permitting increased renal excretion of unconjugated steroids. However, in view of the fact that the largest portion of the urinary steroids is conjugated, the decreased levels of 17-OH-CS indicate a reduction of adrenocortical function in myxedema.

The results of appraisal of adrenocortical function by the 48-hour intravenous ACTH test suggest that the adrenocortical responses of patients with myxedema may be divided into 2 groups (Table 3 and Figure 2). In Group A, the patients exhibited a subnormal response to ACTH stimulation somewhat similar to the response elicited in patients with pituitary in-

sufficiency. That these patients did not have primary pituitary insufficiency is supported by their clinical status, their response to thyroid therapy and the presence of significant levels of urinary gonadotropin. Further, the diminished output of steroids in response to ACTH stimulation returned to normal following achievement of euthyroidism in these subjects. In patients of Group A, the poor adrenocortical response may have been due to either primary alterations in the adrenal gland itself or to diminished ACTH secretion resulting from myxedematous changes in the pituitary. It remains to be demonstrated which, if either, of these factors predominated.

In Group B myxedematous patients there was a supra-normal response on the second day of ACTH administration, as shown by the elevated levels of urinary corticoids. Clinically, this group of patients did not appear to differ significantly from those in Group A, and they had the same levels of serum protein-bound iodine and cholesterol. The responses in Group B were normal on the first day of ACTH stimulation and supranormal on the second day of stimulation (Table 3 and Figure 2). It is of interest that in 3 Group-B patients whose urinary 17-OH-CS were measured on the day following cessation of the ACTH test, the values were unusually elevated (Table 4). It is possible that the division of patients into 2 groups may well be an artificial one, since in a larger series of patients the entire spectrum of values from low to elevated might be demonstrated. A possible mechanism for this exaggerated response is a decreased rate of degradation of ACTH. The interpretation of the findings in both hyperthyroidism and myxedema following ACTH stimulation is difficult, and any simple explanation for these complex phenomena at this time would undoubtedly represent an oversimplification.

It would appear from the data of the present study that the influence of thyroid hormones on adrenocortical secretion of steroids results from a combination of the direct effect of the hormones on the adrenal cortex itself and an indirect effect via modification of the secretion or degradation of pituitary ACTH. The precise mechanism must await further investigation.

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URINARY EXCRETION OF 17-KETOGENIC STEROIDS IN NORMAL SUBJECTS BEFORE AND AFTER STIMULATION WITH INTRAMUSCULAR ZINC-ACTH*†

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ABSTRACT

The estimation of 17-ketogenic steroids in human urine has proved a useful means of indirectly measuring the adrenocortical production of cortisone and hydrocortisone. Studies of precision and accuracy indicate that this procedure is practical for routine clinical purposes. The excretion of 17-ketogenic steroids in day and night 12-hour urine samples was determined in a group of 31 normal subjects. The basal 24-hour excretion was 13.1 mg. (S.D. ±5.0) for males and 10.8 mg. (S.D. ±2.9) for females. A diurnal variation was noted. The output of 17-ketogenic steroids was also studied during two consecutive 12-hour periods following single intramuscular injections of Cortrophin-Zinc. The mean response was approximately proportional to the basal output. A maximal response in terms of both magnitude and duration was obtained with doses of 30 to 40 (USP) units. Larger doses produced no significant increase in steroid output nor prolongation of action. Repeated responses of 17-KGS to intramuscular ACTH may vary considerably. Individual differences between subjects or within the same subject on different occasions largely account for these variations, which appear to be correlated with variations in basal excretion from day to day.

IN RECENT years two methods for the routine measurement of urinary corticoids have received extensive study. The first, based upon the color reaction described by Porter and Silber (1), was introduced by Reddy, Jenkins and Thorn (2) in 1952 and later modified (3–5). This is perhaps the procedure most widely used in this country. A second chemical approach reported by Norymberski in 1952 (6) and also modified

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(7–10) is the oxidative fission and removal of the 20:21 carbon side-chain with the subsequent formation of 17-ketosteroids. Those corticosteroids which undergo oxidation with sodium bismuthate and thereby give rise to 17-ketosteroids are termed 17-ketogenic steroids. These may then be quantitated colorimetrically by the conventional Zimmermann technique. Except for a study by Engbring *et al.* (11), the Norymberski procedure has had only limited clinical use in this country, although it has been widely used in Europe.

Both of these methods for estimation of urinary 17-hydroxycorticosteroid excretion have been comparatively appraised by Nabarro and

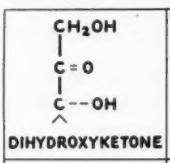	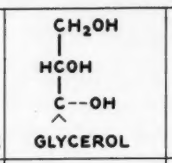	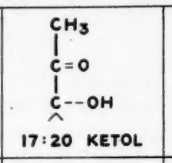	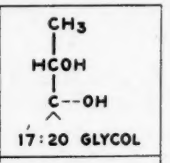
DIHYDROXYKETONE	GLYCEROL	17:20 KETOL	17:20 GLYCOL
CORTISONE CORTISOL	CORTOLONE CORTOL	17 α-HYDROXY-PROGESTERONE	PREGNANETRIOL
+	−	−	−
PORTER - SILBER REACTION (17-OHC)			
+	+	−	+
META-DINITROBENZENE REACTION		AFTER $NaBiO_3$ OXIDATION (17-KGS)	

FIG. 1. 17-Hydroxycorticosteroid groups and side-chain types as determined by Porter-Silber and Norymberski reactions.

Moxham (12, 13) and by Butt *et al.* (14). Both appear to be reliable with regard to accuracy and precision. A major advantage of the Norymberski procedure is that it may be readily adapted to 17-ketosteroid methods already employed by many laboratories.

Chromatographic studies have shown that, like the Reddy method, the Norymberski procedure measures cortisone and hydrocortisone, as well as their tetrahydro derivatives (15). However, these steroids are but one of four known groups of these compounds present in normal human urine. The additional 17-hydroxycorticoids measured by the Norymberski procedure are depicted in Figure 1 according to the types of side-chains. It should be noted that 3α:17α:20α-pregnanetriol is measured as a 17-ketogenic steroid. Though present in only small amounts in normal urine, the excretion of this steroid may be greatly increased in adrenogenital syndromes. Therefore, when the results are properly interpreted, the Norymberski method may also be utilized as a screening procedure for patients with this disorder.

The present report describes the range and variation of urinary 17-ketogenic steroids in normal subjects before and after ACTH stimulation.

MATERIAL AND METHODS

Human volunteers. The subjects consisted of 24 males and 7 females recruited from the house staff, medical students and laboratory personnel of the Los Angeles County General Hospital. The males ranged in age from 19 to 30 years, the females from 21 to 49 years. All were presumably healthy.

Urine collections. Cork-stoppered glass containers with toluene added as a preservative were used throughout the study. Each day was divided into two 12-hour periods and collections were made into separate containers for each twelve hours. Specimens were not refrigerated, since studies performed here have shown that urinary 17-ketogenic steroids are stable for at least one week without refrigeration.

Administration of adrenocorticotropin (ACTH). The proprietary form of ACTH employed throughout was Cortrophin-Zinc (Organon). Though a single lot (No. 1356759A) was used for most of the studies, several other lots were used as well. All injections were given intramuscularly and at approximately the same time for each subject (usually between 8 and 10 A.M.). Two consecutive 12-hour urine collections were then begun for the 24-hour period following each injection.

No subject received ACTH more often than at weekly intervals, in order to avoid potentiation of steroidogenesis from previously given ACTH.

Determination of 17-ketogenic steroids. The method used was virtually that described by Norymberski in 1953 (7). Two aliquots of urine were taken from each specimen. From one, a neutral 17-ketosteroid extract was prepared according to the method of Drekter *et al.* (16). To the second aliquot was added an equal volume of glacial acetic acid. Oxidation with sodium bismuthate was carried out on this aliquot. The bismuthate[1] should be carefully evaluated, since different brands have been shown to cause variations in results (17). The total 17-ketosteroids formed after bismuthate oxidation were hydrolyzed with hydrochloric acid and heat. Extraction was performed, and the neutral 17-ketosteroid fractions from both aliquots were evaporated to dryness under nitrogen at 60° C. The dry residues were taken up with the Zimmermann reagent (meta-dinitrobenzene and alcoholic KOH) to produce the usual color reaction. After completion of the Zimmermann reaction, extraction of interfering chromogens was performed with a methylene chloride-propanol partition. Details of this technique have been published elsewhere (18). Quantitative measurements were made photometrically and reported in terms of dehydroepiandrosterone (DHEA) equivalents. The value for 17-ketogenic steroids was determined by subtraction of the value for 17-ketosteroids in the first aliquot from that for total ketosteroids (after bismuthate oxidation).[2]

RESULTS

Urinary steroids during control period (no ACTH stimulation)

Steroid excretion values obtained on separate day and night 12-hour urine collections are summarized in Table 1. The data are expressed both

[1] The product used in the present study was obtained from the Mathieson Company, Norwood, Ohio.

[2] We are indebted to Dr. Arnold Ware, Professor of Biochemistry and Nutrition, University of Southern California, and Chief Chemist, Los Angeles County Hospital, for the steroid determinations.

in terms of excretion during the day (12-hour) and during the total 24-hour period. Both 17-ketogenic steroids (17-KGS) and 17-ketosteroids (17-KS) were determined on each specimen. Since the data on 17-KS have been extensively reported by others and are not directly pertinent to the present study, only those dealing with 17-KGS are presented in detail.

The *mean 24-hour excretion* of 17-KGS was 13.1 mg. ±5.0 (S.D.) in males, and 10.3 mg. ±2.7 in females. The difference between sexes was statistically significant (P= <0.05).

The *nocturnal decline in urinary excretion* of both 17-ketosteroids and 17-ketogenic steroids was proportionately similar for both sexes. Sixty per cent of the excretion of 17-KGS and 55 per cent of the excretion of 17-KS per twenty-four hours occurred during the daytime 12-hour period for both groups.

Daily fluctuations in the 24-hour output of urinary steroids were studied in 4 male subjects who collected specimens on at least five consecutive days. The *maximum* deviation observed in these subjects was ±3.5 mg. for both 17-KGS and 17KS. In 16 of the total group of 24 males, more than one baseline 24-hour urine collection was made. The mean daily spontaneous variation was ±2.8 mg. for 17-KGS and ±1.8 mg. for 17-KS. On

TABLE 1. URINARY EXCRETION (MG.) OF 17-KETOGENIC STEROIDS (17-KGS) IN NORMAL MALE AND FEMALE SUBJECTS: BASAL LEVELS AND RESPONSE TO SINGLE INTRAMUSCULAR DOSES OF ACTH (CORTROPHIN-ZINC)

Dose of ACTH	Subjects		No. of studies*	Mean		Range				Standard deviation (±)		Standard error of mean (±)	
						Low	High	Low	High				
	No.	Sex		day** 12 hrs.	24 hrs.†	day 12 hrs.		24 hrs.		day 12 hrs.	24 hrs.	day 12 hrs.	24 hrs.
None (basal excretion)	24	M	116	7.8	13.1	3.0	16.6	7.0	29.7	3.3	5.0	0.7	1.0
	14	F	61	6.9	10.3	4.2	9.9	6.0	16.9	2.2	2.7	0.8	0.7
20 units	16	M	51	13.2	20.2	5.5	47.4	10.0	51.4	9.5	10.1	2.4	2.5
	3	F	5	8.8	8.8‡	4.0	15.8	6.5	11.2	6.2	2.4	3.6	1.4
30 units	19	M	95	17.6	29.3	9.8	38.2	15.2	63.8	7.6	11.2	1.7	2.5
	3	F	12	10.0	19.0	9.8	10.3	13.3	26.0	2.9	6.4	1.7	3.8
40 units	22	M	85	18.1	29.2	5.0	28.6	19.6	42.8	5.5	6.9	1.2	1.5
	12	F	39	16.3	24.7	10.4	20.0	13.3	33.1	4.1	6.3	1.6	1.8
50 units	10	M	28	18.7	29.8	11.5	29.6	22.1	43.2	5.0	7.1	1.6	2.2
	3	F	8	13.8	20.4	3.4	23.4	10.0	29.4	10.0	9.7	5.8	5.6
60 units	14	M	33	19.3	31.6	13.3	28.7	21.7	40.6	4.9	7.2	1.3	1.9
	2	F	4	9.9	15.5	7.1	12.7	9.7	21.3	—	—	—	—

* Two successive 12-hour urine specimens in each study.

** Daytime 12-hour urine collection period.

† Sum of day and night 12-hour collection periods.

‡ One subject did not collect a specimen during the second 12-hour period.

the basis of limited observations, no correlation was noted with changes in urine volume, nor was any seasonal fluctuation observed.

Urinary steroids following ACTH stimulation

Group characteristics. One hundred and eighty-seven injections of Cortrophin-Zinc were given at dosage levels of 20, 30, 40, 50 and 60 (USP) units. Two successive 12-hour urine specimens were collected after each injection, except in a few cases in which only one specimen was obtainable. The results are summarized in Table 1.

Males and females exhibited certain similarities in steroidogenic response. The dose-response curve for each sex rose sharply between doses of 20 and 30 units. The response after 40 units appeared to represent maximum stimulation, since larger doses produced no further increase in 17-KGS output (Table 1).

A difference between sexes was noted in the total quantity of 17-KGS excreted during the 24-hour period following 40 units of ACTH. When expressed independently of basal levels, *i.e.*, as milligrams of 17-KGS per day, the males had a greater maximum response ("adrenocortical reserve" or "capacity") to single intramuscular doses of zinc-ACTH than did females ($P = <0.02$) (Table 1). However, though the level of maximum response may be related to sex, this relationship may be an indirect one which reflects the differences in basal levels already noted between the two groups. When the response was correlated with basal output, considerable overlapping was observed between sexes. It would appear that resting secretory activity influences adrenocortical reserve to a greater extent than does sex (Fig. 2).

In both groups the secretion of 17-KS was uniformly less affected than

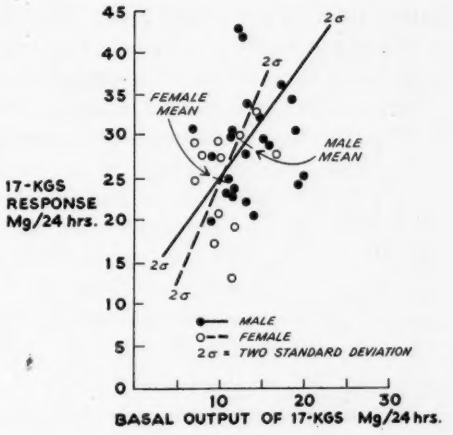

FIG. 2. Relationship of basal output of 17-KGS to response after 40 units of Cortrophin-Zinc intramuscularly, in male and female subjects.

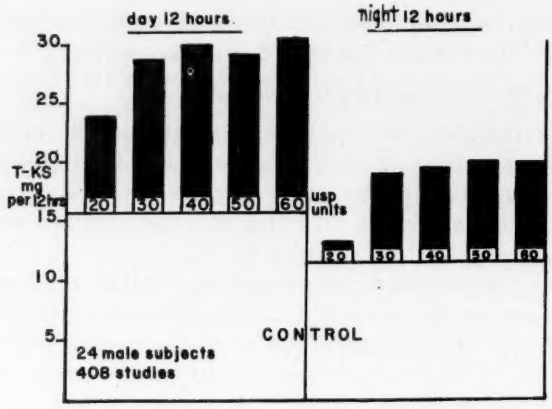

FIG. 3. Dose-response pattern during consecutive 12-hour periods after single intramuscular doses of Cortrophin-Zinc. (Corticoid output is plotted as "T-KS," the arithmetic sum of 17-KS and 17-KGS. The columns indicate the rises above day and night basal levels respectively after increasing doses of ACTH.)

that of 17-KGS by the administration of zinc-ACTH. Following 40 units, males excreted 20.3 mg. $\pm$5.8 (S.D.) and females 16.6 mg. $\pm$3.0 per twenty-four hours.

The duration of action of the intramuscular ACTH preparation used in the present study was indicated by the excretion of 17-KGS during the two consecutive 12-hour urine collection periods following single injections. At each dosage level studied, the material exerted its major effects during only the first twelve hours following injection. Duration of effect appeared to be unrelated to dosage. During the second 12-hour period following injection, steroid production could not be significantly increased when doses larger than 30 units were given (Fig. 3). Thus, maximum doses resulted in a plateau in terms of magnitude as well as duration of response. Submaximal doses (*i.e.*, 20 units) appeared to expend most of their steroidogenic effect within the first twelve hours after administration.

The duration of induced steroidogenesis may not only be influenced by the preparation and dosage of exogenous ACTH, but also by basal secretory activity. When males and females were compared it was found that during the first twelve hours after injection of zinc-ACTH the excretion of 17-KGS each group rose above baseline (daytime 12-hour) levels by approximately 135 per cent. However, the excretion of 17-KGS during the second 12-hour period differed according to sex. During this period the 17-KGS output of males fell to 62 per cent above the night basal output, whereas that of the females dropped significantly more—to 41 per cent above the control level. (The difference of 21 per cent between these two groups was approximately that previously noted between their basal lev-

els.) This would suggest that subjects with low basal outputs of 17-KGS tend to respond to intramuscular ACTH for shorter periods of time.

Individual variations in response to ACTH. By means of repeated testing, several factors which influence the response to intramuscular zinc-ACTH were evaluated (Table 2).

TABLE 2. VARIABLES WHICH INFLUENCE RESPONSE TO INTRAMUSCULAR ACTH (CORTROPHIN-ZINC)

Variable	Subjects	ACTH		Test data—17-KGS excretion (mg./24 hrs.)				
		Dose	Lot					
Subjects	"Consistent responders"*			Subject	J.K.†	F.L.	I.G.	R.E.
	varied	30	same	Test #1	29.1	17.6	25.5	12.4
				Test #2	25.6	17.1	28.8	16.6
	"Random responders"			Subject	D.K.	C.R.M.	L.G.	D.W.
	varied	30	same	Test #1	21.2	38.5	31.3	45.2
				Test #2	12.0	11.4	17.0	24.8
Dosage				Trial	#1	#2	#3	#4
	D.W.	20	#1356		18.4	17.0	18.0	15.7
	D.W.	30	#1356		45.2	24.8	—	—
	D.W.	40	#1356		34.3	26.0	14.2	—
	D.W.	50	#1356		41.2	45.3	—	—
	D.W.	60	#1356		34.1	—	—	—
Potency of ACTH lot					ACTH dose			
					30 units		40 units	
				Subject	J.K.	F.L.	I.G.	R.E.
	same	same	#1356		16.9	16.1	26.1	24.9
			#1356		33.7	37.2	33.2	29.2
					ACTH dose			
				Basal	30	40	50	60
	F.L.	varied	#1356	21	16.1	27.5	24.9	42.3
			#1356	19.3	37.2	33.3	27.8	—

* Subjects whose responses were within 5.0 mg. per day of each other after at least one repetition of the test with the same dose and lot of Cortrophin-Zinc.

† Tested with 40 units.

Certain subjects ("consistent responders") were relatively stable and excreted approximately the same quantity of 17-KGS when the same stimulus was repeated. In other subjects the responses were quite variable. Except by repeated testing, no criteria were apparent for differentiating subjects with consistent responses from those whose responses were variable. Furthermore, as demonstrated by 1 subject (D.W.), despite an observed consistency in response at one dosage level, repeated administration of larger doses of the same lot of ACTH elicited responses which were quite unpredictable (Table 2). The extent of the variations possible between *different subjects* was illustrated in 17 of the males given the same lot (#1356) and dose (40 units) of Cortrophin-Zinc. The 17-KGS response ranged from 19.6 to 42.8 mg. per twenty-four hours.

Variations in potency between two lots were compared in subjects selected for consistency of response. One lot (#1156) repeatedly resulted in a greater excretion of 17-KGS than the other (Table 2). When given to Subject F.L., a dose of more than 50 units of lot #1356 was required to produce a twofold rise above the baseline excretion, whereas a dose of only 30 units (or less) of lot #1156 was needed to elicit a comparable response.

The clinical use of intramuscular ACTH is often limited, for practical reasons, to the use of random lots of the hormone. The variation in 17-KGS response which may be anticipated when random lots are given to the same subject is indicated in Table 3. It is noteworthy that the percentage deviation between repeated responses was proportional to the fluctuation in basal excretion. Thus, in Subject H.R., both the mean output of 17-KGS *and* the average deviation from the mean (before ACTH) rose twofold following 40 units of Cortrophin-Zinc.

The use of a single lot of ACTH, as opposed to random lots, does not significantly reduce variations in responsiveness. When 5 males were given 40 units from *random lots* of Cortrophin-Zinc, the 24-hour excretion of 17-KGS ranged from 1.7 to 4.3 times the baseline level. After administration of a *single* lot (#1356) to 12 subjects the range observed was 1.8 to 4.4 times above baseline. When 3 subjects (H.R., J.K. and W.G.) received repeated injections from a *single* lot (#1356), the mean variation of values between trials with the same dose was ± 15 per cent. As indicated in Table 3, when random lots were used in these subjects the 17-KGS response showed an average deviation of ± 17 per cent. Thus, individual variations, whether between different subjects or in the same subject on different occasions, appear to have a greater influence on the response than does the lot of ACTH used.

Certain apparently normal subjects demonstrated an unusual degree of variation in response to intramuscular ACTH. Of these, 4 appeared to be "hyper-responsive" on various occasions (Table 3). In this group the

TABLE 3. COMPARISON OF VARIATIONS IN BASAL EXCRETION OF 17-KGS WITH VARIATIONS IN RESPONSE TO INTRAMUSCULAR ACTH (CORTROPHIN-ZINC)

	Subject	Control			ACTH			
		No. of studies	Mean 17-KGS (mg./24 hrs.)	Deviation from mean* (%)	Dose	No. of studies	Mean 17-KGS (mg./24 hrs.)	Deviation from mean (%)
Normal	H.R.	8	15.0	± 8	40†	5	29.9	±16
	J.K.	8	12.8	±10	30†	8	21.4	±23
	W.G.	3	15.4	± 5	30†	3	30.6	±12
	Mean	—	14.4	± 8	—	—	27.3	±17
Hyper-responsive	M.W.	2	29.7	±54	20	1	51 4	
					30	1	49.3	
	D.H.	3	17.5	±29	30	1	63.8	
	C.R.M.	2	18.8	±36	40	1	27.3	
					40	1	42.2	
	D.W.	2	10.8	±33	30	1	45.2	
					30	1	24.8	
					Dose			
					20	30	40	50	60
Hypo-responsive	J.L.	2	19.9	± 3	11.3	23.4	24.6	26.0	23.3
	F.L.	2	20.7	± 0	17.6	16.1	27.5	24.9	42.3
	E.G.	2	19.4	± 5	13.2	9.6	21.3	32.3	25.0

* The average variation observed when each of the studies on a single subject was compared with the mean for that subject.

† Each subject received the same dose from random lots of Cortrophin-zinc.

basal excretions of 17-KGS were at the upper limits of normal and there was a marked fluctuation between baseline values. A second group of 3 subjects appeared to be relatively "hypo-responsive" to intramuscular ACTH. They also exhibited a level of basal secretory activity which was at or above the upper limits of normal. However, in contrast to the "hyper-responsive" group, the baseline fluctuation in this group was slight, and appeared to be even more constant from day to day than that in the subjects with normal responses.

DISCUSSION

The present study does not attempt a complete evaluation of the technical aspects of 17-ketogenic steroid determination. Pertinent data in this regard have been furnished by Sobel and Golub *et al.* in recent reports describing the laboratory procedure in detail (19, 20). Based on results of work carried out at this center, the expected laboratory error of the method would appear to be small enough to allow for the use of the technique for routine purposes. A precision study of 16 "blind" duplicates (from the same urine specimens) showed an average difference of ± 1.8 mg. (total 17-ketosteroids) between samples. This is in agreement with results of a similar study by Diczfalusy *et al.* who reported a difference of ± 1.6 mg. per twenty-four hours between duplicates (15). Butt *et al.* reported the precision of this method as ± 7.2 per cent, based upon duplicate studies (14).

On the basis of recovery studies, the Norymberski procedure compares satisfactorily with other routine methods of measuring urinary corticoids. When cortisone or hydrocortisone was added to urine, between 70 to 90 per cent of the added steroid was recovered. Comparable recoveries have been reported by Nabarro (13), Sobel (19), Birke (21) and their associates.

These factors, as well as the relative simplicity of the procedure, account for the close range of agreement reported from widely separate centers for the daily excretion of 17-ketogenic steroids by normal subjects (7, 13, 15, 21–24). The data on normal basal values presented in this report are in close conformity with the data of these previous studies.

Results of the present study indicate that in normal subjects the excretion of 17-ketogenic steroids reflects changes in adrenocortical function which are similar to those previously described in terms of Porter-Silber chromogens. The observed diurnal pattern in 17-ketogenic steroid output has been reported for 17-hydroxycorticoids by Laidlaw and co-workers (25) and for 17-ketosteroids by Pincus (26). The observed influence of sex differences on basal urinary corticoid excretion was similar to that determined by other methods (2, 27). It is of interest that males excreted approximately 20 per cent more 17-KGS than females. This agrees with the report of Levell *et al.* who noted that the excretion in males exceeded that in females by 22 per cent (24). Borth and associates observed a 17-KGS difference between sexes of 30 per cent. They related the greater basal 17-KGS excretion in males to greater body weight and height, but attributed the greater 17-KGS excretion to the testicular contribution (22).

A relatively small, though significant, daily fluctuation in 17-KGS output was noted. A similar narrow daily fluctuation unrelated to urine volume has been reported for 17-hydroxycorticosteroids by Sandberg *et al.* (27). Though no pronounced seasonal changes were noted in the present

study, a marked decrease in basal 17-KGS excretion has been observed during the summer months in the tropics (28).

It is of interest that the basal daily variation was greater in males (21.4 per cent) than in females (12.7 per cent). For the entire group, the mean was not as high as the 27 to 48 per cent daily fluctuation reported by Prunty (29), but more nearly approximated the 18 per cent noted by Levell *et al.* (24). It is likely that occupational differences between sexes, as well as among populations sampled, may account for the differences in day-to-day fluctuations in adrenocortical activity.

Following stimulation with ACTH, the urinary output of 17-KGS is a more sensitive index of adrenocortical activation than is the 17-KS output. A similar greater response of Porter-Silber chromogens compared to the response of 17-KS has previously been reported (30).

The data obtained following various single intramuscular doses of a long-acting ACTH preparation suggest certain dose-response relationships. It is apparent that the preparation used elicited a plateau of *maximal* steroidogenic response only during the initial 12-hour period following injection. This duration was in agreement with that of the 17-hydroxycorticoid blood levels observed by Geller and associates following the intramuscular administration of 40 units of Cortrophin-Zinc (31). In a similar study, Siegel *et al.* also estimated the duration of action of this material as being from eight to sixteen hours (32).

The correlation between the basal output of urinary 17-KGS and the output after intravenous ACTH was previously noted by Birke *et al.* (21). The same workers also noted that this correlation was independent of sex. These observations, supported by the present findings with *intramuscular* ACTH, are consistent with the hypothesis that basal secretory activity and response to ACTH are both proportional to adrenocortical mass. This in turn seems to be related to certain body characteristics such as surface area, height and weight (22).

The plateau above which larger doses no longer increase the production of 17-hydroxycorticoids has been investigated by Forsham *et al.* (33) and by Eik-Nes *et al.* (34) with *intravenous* ACTH. Based on the results of these studies, the dosage of circulating ACTH required to produce the maximal response is in the range of from 4 to 15 USP units. Our findings indicate that between 30 and 40 USP units of Cortrophin-Zinc are required to induce a maximal response following a single *intramuscular* injection. The use of larger doses, *e.g.*, 50 USP units, elicits no greater response by either intravenous (34) or intramuscular routes.

Normal subjects may show wide variations in 17-KGS excretion when repeatedly tested intramuscularly with the same dose and lot of ACTH.

This cannot be attributed to the intramuscular route of administration alone, since similar variations occur when intravenous stimulation is used (33, 34). The correlation of 17-KGS response with the basal 17-KGS output may partially account for the range of values observed. It should be pointed out, however, that basal secretory activity may be quite variable on "control" and on "test" days. The desirability of using more than one 24-hour urine collection to establish baseline output is evident.

In order to establish whether or not a normal subject is "hyper-" or "hypo-responsive" to intramuscular ACTH, repeated testing and observation may be necessary. Similar aberrant responses to intramuscular ACTH in presumably normal persons have been described previously (35). The finding of the present study that subjects who responded excessively also exhibited wide fluctuations in basal secretion of 17-KGS suggests either a greater than normal, though transient, adrenocortical sensitivity to exogenous ACTH, or marked daily variations in endogenous ACTH secretion. In contrast, the subjects who were relatively insensitive to intramuscular ACTH maintained a persistently high basal excretion of 17-KGS from day to day. This may also have been a reflection of a somewhat excessive production of endogenous ACTH which resulted in maintenance of steroidogenesis at almost maximal levels and a decrease in response when Cortrophin-Zinc was administered to these subjects.

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STRUCTURE-ACTIVITY RELATIONSHIPS OF ANABOLIC STEROIDS: ROLE OF THE 19-METHYL GROUP

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ABSTRACT

Anabolic agents are therapeutically effective in a variety of diseases, and dissociation of the anabolic from undesirable androgenic activity has been shown in several newer steroid derivatives. One such modification is removal of the C-19-methyl group producing 19-nor compounds. With adequate animal data now available, a critical comparative clinical study was essential for evaluating the applicability of these structure-activity relationships to man. Since in the animal studies 17α-ethyl-19-nortestosterone had optimal anabolic activity, all possible compounds with a two-carbon atom side-chain at the 17α-position were studied, *viz*, 17α-ethyl-, 17α-vinyl-, and 17α-ethynyl derivatives of the testosterone (19-methyl) and 19-nortestosterone series. Each 19-nor compound was compared with the analogous 19-methyl compound (the only structural difference between pair members being presence or absence of the 19-methyl group) and with 17α-methyltestosterone. Comparisons were made at two dosage levels in the same person. Subjects were normal young women maintained on constant diets in a metabolic ward. Urinary nitrogen, sodium and potassium, and body weight were measured daily. Compounds of the 19-methyl series were inactive at dosage levels of 100–200 mg. per day, orally. All 19-nor compounds were strongly active in producing nitrogen retention, showing anabolic activity equivalent to that of 17α-methyltestosterone by this assay procedure. The high clinical potency of 2 of these, 17α-vinyl- and 17α-ethynyl-19-nortestosterone, could not have been predicted, since they manifested very low activity in animal studies. The observations suggest certain general relationships between structure, anabolic and androgenic activities of steroids.

THE use of drugs to produce nitrogen retention in man is increasing. Studies reported here were undertaken to evaluate the potency of several such new anabolic compounds, and to elucidate structure-activity relationships.

Replacement of the angular methyl group at the C_{10} position of the steroid nucleus by hydrogen yields compounds having favorable anabolic ac-

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tivity in comparison to their androgenicity (1–6). These compounds are members of the 19-nor series of steroids, *i.e.*, those lacking carbon atom number 19, which is attached at C_{10}. Since removal of the methyl group at C_{10} produces this desirable change in the spectrum of activities, it seemed logical to compare 19-nor steroids with corresponding compounds in which the 19-methyl group was intact.

Drugs that are effective when given by mouth are preferred. Since alkyl substitution at C_{17} confers oral activity, compounds with this structural feature were selected for screening. Studies in animals had shown that 17α-ethyl-19-nortestosterone has optimal anabolic activity relative to its androgenicity (7, 8). Therefore all possible variations in two-carbon atom side-chains substituted at the 17α position were compared. The substituents were a) ethyl, b) vinyl, and c) ethynyl. The compounds were assayed in pairs, the structural difference between the pair members being presence or absence of the 19-methyl group. Thus, 17α-ethyltestosterone was compared with 17α-ethyl-19-nortestosterone, both being given at two dosage levels to the same subject. Steroids showing activity were also compared at two dosage levels with the potent anabolic agent, 17α-methyltestosterone in a second subject.

SUBJECTS AND METHODS

The subjects were 6 normal volunteer women ranging in age from 19 to 34 years. Physical examination and routine laboratory tests revealed no evidence of significant disease. They were maintained with self-selected constant metabolic diets and remained in an air-conditioned environment throughout the period of study, with the exception of Subjects J.W. and E.P. All drugs[1] were given for four-day periods and were administered orally in four equal fractional doses spaced over the waking hours. On the first day, the initial dose was withheld until blood was drawn. The final dose was always given by 11:00 P.M. on the fourth day. A constant level of moderate physical activity comparable to that of a laboratory worker was maintained throughout the studies. After three days of the constant diet, urine was collected in 24-hour specimens for determination of nitrogen by a modified micro-Kjeldahl method (9), and of sodium and potassium by internal standard flame photometry.

Serum sodium, calcium (by flame photometry), inorganic phosphorus, blood urea nitrogen, cholesterol and fasting blood sugar levels were obtained at the start of each four-day period. As anticipated, there were no significant changes in the values of any of these constituents during the studies, and since all data were in the normal range, they are not reported here.

RESULTS

Clinical

All of the compounds were well tolerated orally, and subjective symptomatology was present only in the 2 subjects (S.D. and E.K.) receiving

[1] We wish to thank Dr. John Mote and Syntex, S. A., Mexico City, for generously supplying all drugs used in these studies.

17α-vinyl-19-nortestosterone. The feeling of "having the jitters" began on the second day and continued throughout the period of drug administration. Both subjects showed moderate tremors of the extended hands. BMR measurements two days after the course of steroid were −8 per cent (Subject S.D.) and −16 per cent (Subject E.K.) Icterus (10, 11) was not noted during the short periods of drug administration employed here.

17α-Ethyl compounds

Data comparing 17α-ethyl-19-nortestosterone with the corresponding 19-methyl compound in Subject N.B. are charted in Figure 1. (The equili-

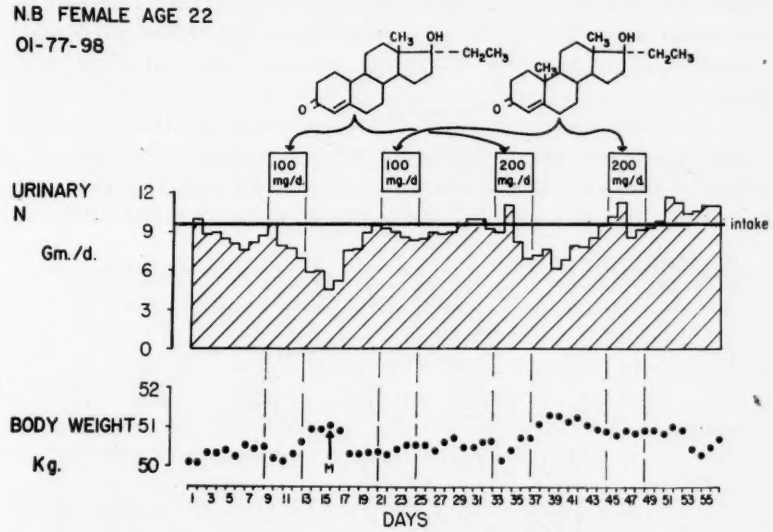

FIG. 1. Effect of 17α-ethyl-19-nortestosterone and 17α-ethyltestosterone on urinary nitrogen and body weight. In this and the succeeding figures the following conventions are used: 1) the compounds tested (shown at the top) were given alternately (as shown by the arrows) for four-day periods (designated by the interrupted vertical lines) at the dosages inscribed in the blocks; 2) in the middle of the chart, the nitrogen intake (Gm./day) is represented as the ordinate charted upward from the 0 baseline to the heavy intake line; 3) daily urinary nitrogen excretion (Gm./day) is also charted upward as the ordinate from the same 0 baseline, and comprises the hatched area; 4) nitrogen retention is therefore shown by the clear area below the intake line and above the hatched area; 5) when charted, sodium and potassium intake, excretion and retention are represented in the same way; 6) daily body weights (on a metabolic scale) are charted at the bottom; and 7) M designates the first day of the menstrual cycle.

Note the marked fall in urinary nitrogen excretion associated with administration of the 19-nor compound. (The 19-methyl compound was virtually inactive.) A rise in body weight is seen with administration of the 19-nor compound, but not with the 19-methyl derivative.

bration period during the three days of constant diet is not shown.) The drugs were given alternately, as shown by the arrows—first at a dosage level of 100 mg. per day, then at 200 mg. per day for four-day periods. There was an eight-day control period between successive courses of drugs. A high level of nitrogen retention was observed with the 19-nor compound, but there was no activity with the 19-methyl derivative. The decrease in urinary nitrogen excretion was evident on the second to third day of treatment and continued for six to seven days after treatment was stopped. Since there was no more nitrogen retention (measured by fall in urinary nitrogen excretion) when the dose of the 19-nor compound was doubled, it is evident that the dose-response curve reached a plateau at the lower dosage level. The potent 19-nor derivative produced a 0.5-Kg. gain in weight, but the inactive 19-methyl compound had no effect on body weight.

Data on a second subject (B.B.) in whom 17α-ethyl-19-nortestosterone was compared with 17α-methyltestosterone are charted in Figure 2. The schedule of drug administration was the same as that in Figure 1. Both drugs caused a comparable fall in urinary nitrogen excretion at these dos-

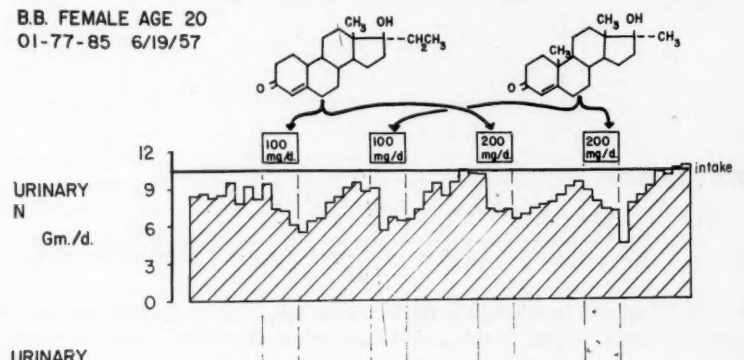

FIG. 2. Effect of 17α-ethyl-19-nortestosterone and 17α-methyltestosterone on urinary nitrogen and body weight. Both compounds caused an equal and marked fall in nitrogen excretion and comparable increases in body weight. Suppression of urinary sodium excretion is shown for the 19-nor compound.

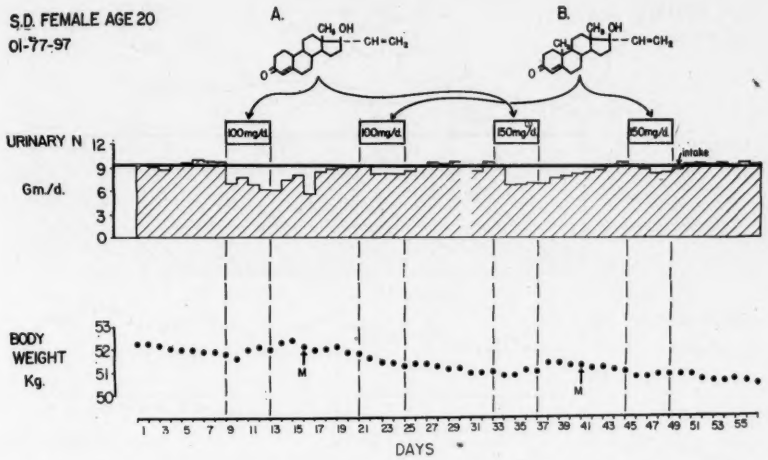

FIG. 3. Effect of 17α-vinyl-19-nortestosterone and 17α-vinyltestosterone on urinary nitrogen and body weight. Note the marked fall in nitrogen excretion (and increases in body weight) associated with administration of the 19-nor compound.

age levels. The onset of action was on the second day for both drugs and the effect persisted for five to seven days with the 19-nor compound. The duration of action of 17α-methyltestosterone appeared to be somewhat shorter at these dosage levels. A fall in urinary sodium excretion was seen during, and for three days after giving the 19-nor compound, and some of the gain in weight was attributable to gain in extracellular fluid. Both compounds caused about the same gain in weight, independently of the dose.

17α-Vinyl compounds

In Figure 3 are data comparing 17α-vinyl-19-nortestosterone with the corresponding 19-methyl compound in Subject S.D. The same schedule of drug administration was employed here as previously, but the higher dosage level was half again, rather than double the lower dosage. In this subject also, the 19-nor compound caused a decrease in urinary nitrogen excretion, whereas the 19-methyl compound was inactive. Total nitrogen retention was independent of the dosage at this level. Retention began promptly when the drug was given, and continued for four days after the last dose. A gain in weight, persisting for two days after stopping the drug, occurred with the 19-nor compound.

In Figure 4 are data comparing 17α-vinyl-19-nortestosterone with 17α-methyltestosterone in another subject (E.K.). Dosage levels corresponded with those used in the previous study (Fig. 3). The 19-nor compound appeared to be of the same order of potency as 17α-methyltestosterone in

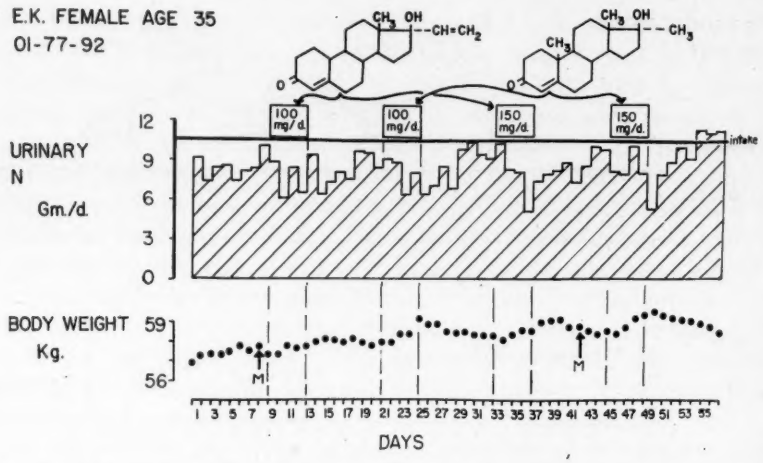

FIG. 4. Effect of 17α-vinyl-19-nortestosterone and 17α-methyltestosterone on urinary nitrogen and body weight. Both compounds produced an equal fall in nitrogen excretion and comparable increases in body weight.

causing nitrogen retention. A gain in weight (perhaps somewhat greater with 17α-methyltestosterone) occurred with both compounds.

17α-Ethynyl compounds

Data comparing 17α-ethynyl-19-nortestosterone with the corresponding 19-methyl compound in Subject E.P. are presented in Figure 5. Urinary nitrogen excretions indicate that the 19-nor compound caused nitrogen retention at both the 100-mg. and 200-mg. daily dosage levels. The 19-methyl compound was inactive. A weight gain of 700 to 800 Gm., reaching its maximum on the third to the fourth day after the last dose, occurred with the 19-nor compound.

Figure 6 compares 17α-ethynyl-19-nortestosterone with 17α-methyltestosterone in Subject J.W. The comparison was made at the higher dosage level first. The drugs induced comparable nitrogen retention, as measured by the fall in urinary nitrogen excretion. Nitrogen retention persisted for four to six days after stopping the medication. A fall in urinary sodium excretion occurred by the third day after starting the drugs and continued for two to four days after stopping them. Comparable weight gains of the order of 1 Kg. were seen with both drugs at the two dosage levels, at a time corresponding to the suppression of urinary sodium excretion. Gain and loss of weight occurred more abruptly with 17α-methyltestosterone than with the 19-nor compound. Urinary potassium excretion fell during the periods of nitrogen and sodium retention induced by these drugs. As

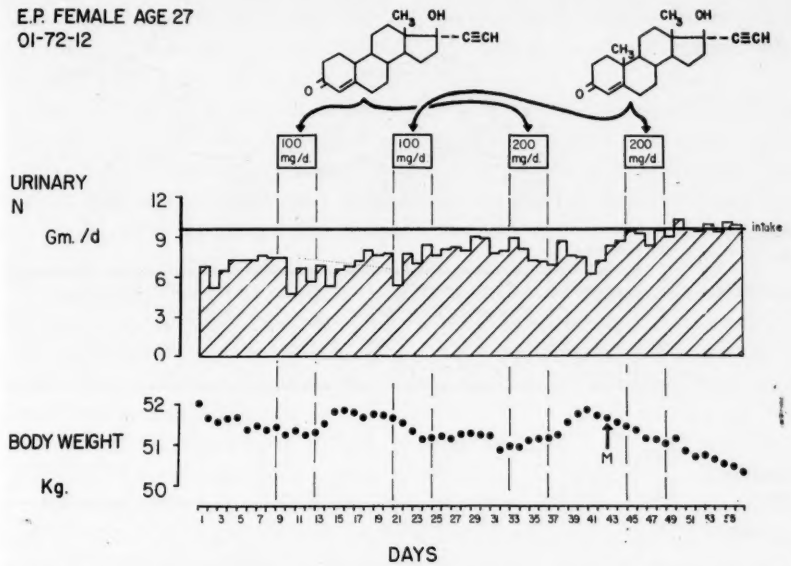

FIG. 5. Effect of 17α-ethynyl-19-nortestosterone and 17α-ethynyltestosterone on urinary nitrogen and body weight. Note the fall in nitrogen excretion (and increase in body weight) associated with administration of the 19-nor compound.

judged from changes in urinary excretion alone, the ratio of potassium to nitrogen retained was far greater than that expected on the assumption that "protoplasm" was deposited (12). (The average potassium to nitrogen ratio was 11 instead of 2.7.) This was noted by other investigators with 17α-ethyl-19-nortestosterone (13, 14).

In this study, a twelve-day period intervened between successive courses of drugs, as compared with the eight-day interval in the previous studies. The data show an adequate return to baseline levels by the eighth day after the last dose of drug.

DISCUSSION

These data demonstrate an interesting structure-activity relationship: in Δ^4-3-keto steroids with a two-carbon atom side-chain at position 17, removal of the 19-methyl group confers pronounced anabolic activity on a molecule previously inactive or only slightly active. This relationship of the 19-methyl group to physiologic activity of steroids appears to be a general one. Δ^4-3-Keto-compounds of both the 19-nor and 19-methyl series have the same spectrum of physiologic activities; removal of the 19-methyl group enhances certain activities and either does not alter or depresses others (1–8).

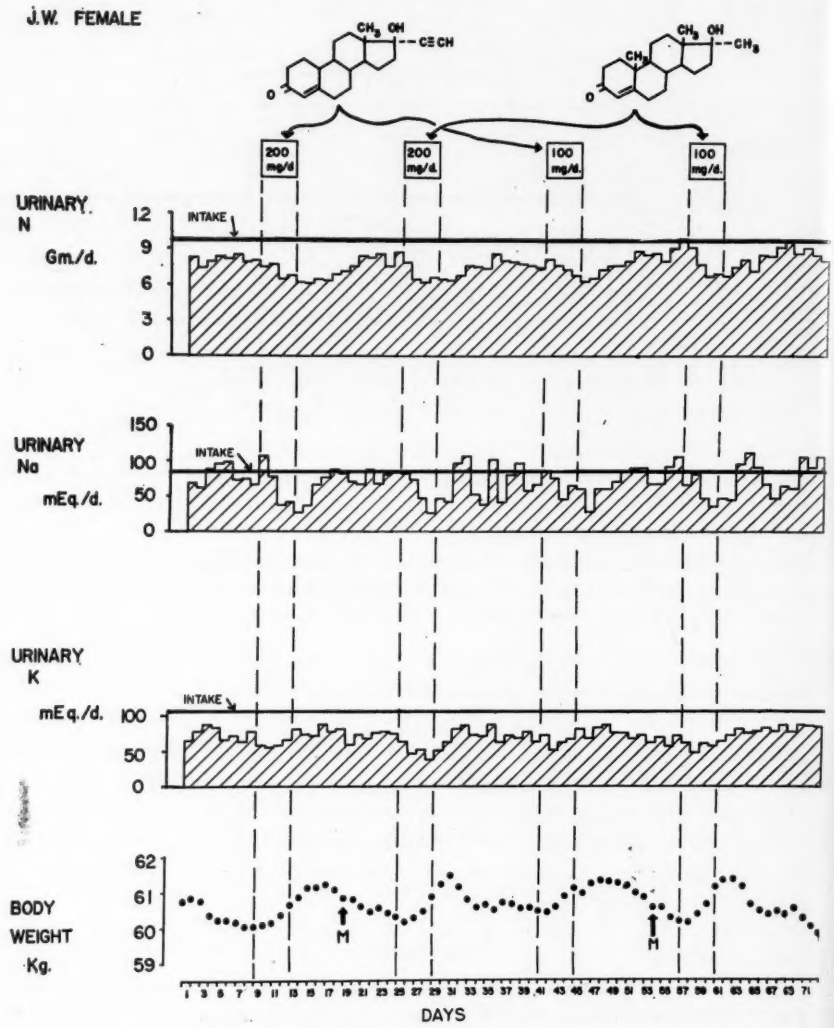

FIG. 6. Effect of 17α-ethynyl-19-nortestosterone and 17α-methyltestosterone on urinary nitrogen, sodium and potassium, and body weight. Both compounds produced marked and nearly equal falls in nitrogen excretion and comparable increases in body weight.

All of these compounds have already had at least a preliminary evaluation for anabolic activity, the findings being generally consonant with those recorded here.

17α-Ethyltestosterone was found to be inactive in immature dwarf boys when given at a dosage level of 20–40 mg. per day both orally and parenterally (15). It has been reported to be "a very weak anabolic agent" (16), and data from studies in animals indicate that it has one tenth the myotropic and one twentieth the androgenic activity of testosterone propionate (17).

17α-Vinyltestosterone in large doses manifested weak myotropic and androgenic activities in castrated male rats (1) and proved to be ineffective in the therapy of patients with metastatic breast cancer, in whom it had little or no virilizing effect at a dosage level of 100 mg. intramuscularly three times a week (18).

17α-Ethynyltestosterone failed to produce anabolic or androgenic effects in immature dwarfs in a dosage of 40 mg. a day by mouth (19). An oral dosage of 120 mg. per day for eighteen days failed to produce nitrogen retention in a 50-year-old woman with Cushing's syndrome (20). In an 8-year-old girl with Addison's disease, however, 90 mg. per day given orally produced nitrogen retention comparable to that following a similar dose of 17α-methyltestosterone (21). In contrast, at a level of 300 mg. per day orally, 17α-ethynyltestosterone was catabolic in 2 subjects and without effect on nitrogen balance in a third (22). This was not confirmed in 3 subjects given the drug orally (23). In an elderly man given 200 mg. per day for four days, followed by 300 mg. per day for six days, the data on urinary nitrogen indicated a slight anabolic response. No discernible effects on protein metabolism were produced by 300 mg. per day in a postmenopausal woman, nor by 60 mg. per day in a third subject.

Thus, all available data support our finding that the 19-methyl compounds are inactive or have a low level of anabolic activity.

The 19-nor series of compounds has recently been developed by partial synthesis and is being extensively studied for both anabolic and progestational activity.

17α-Ethyl-19-nortestosterone is the compound that has been most intensively investigated as an anabolic agent. The high potency demonstrated by studies in animals (7, 8, 24, 25) has also been found in clinical investigations. At a dosage level of 50–100 mg. per day orally or intramuscularly it has produced prompt, significant nitrogen retention in patients, and the data suggest that 50 mg. per day would have the maximum effect (14). The fall in plasma inorganic phosphorus and alkaline phosphatase concentration, and the irregular variation in plasma calcium concentration reported in these patients, most of whom had gastric ulcer, were not

found in our study with normal subjects, although the increases in body weight were comparable. 17α-Ethyl-19-nortestosterone (50 mg. per day intramuscularly) induced positive nitrogen, phosphorus and potassium balances in another group of patients, as shown by the fall in urinary excretion of these elements (26). The increases in body weight were also comparable to those observed in the present study. No clinical or metabolic effects could be detected in 2 of these patients when receiving 25 mg. per day intramuscularly for ten days. There were no changes in the level of serum alkaline phosphatase. In another series, a dosage of 25 mg. per day given intramuscularly to an elderly patient was anabolically as active as testosterone propionate, producing a positive nitrogen balance averaging 2 Gm. daily (23). Nitrogen retention and a gain in weight comparable to that found in the present studies occurred in a group of cardiac patients given only 25–75 mg. per day (27). In a group of chronically underweight subjects, 25–50 mg. per day orally resulted in a weight gain averaging 2 pounds per month—significantly above that of the control subjects (28). In a dosage of 25 mg. per day intramuscularly, combined with a high caloric and protein intake, 17α-ethyl-19-nortestosterone decreased the negative nitrogen balance produced by major surgery to below that of control subjects not receiving the drug (29).

17α-Vinyl-19-nortestosterone has little anabolic activity in animals (7, 30). It had not been evaluated clinically as an anabolic agent up to the time of the present study.

17α-Ethynyl-19-nortestosterone at a dosage level of 40 mg. per day orally has induced a positive nitrogen balance (2 Gm. per day) in an elderly man (23). This high degree of anabolic activity was accompanied by moderate sodium retention. In animals, the compound was shown to possess little anabolic activity (7) by the levator ani test, but considerable activity in maintaining hepatic weight and nitrogen during starvation (30). It also produced weight gain and a subjective feeling of well being in normal men, comparable to the effect of 17α-ethyl-19-nortestosterone in a similar group of normal subjects. Clinically, the effects of 17α-ethynyl-19-nortestosterone were so similar to those of the 17α-ethyl compound that a comparable degree of anabolic activity was inferred by the investigators (31). It is surprising that 17α-ethynyl-19-nortestosterone is estrogenic, rather than androgenic, when tested in animals (7) and man (32).

Thus, available data support our findings to the effect that removal of the 19-methyl group confers a high degree of anabolic activity on 17-ethyl- and 17-ethynyl-testosterone. This study shows that it has a similar effect on 17-vinyltestosterone. The finding that 17-vinyl and 17-ethynyl derivatives of 19-nortestosterone are highly potent anabolic agents in man is surprising in view of their low activity in animals.

It is interesting to compare the anabolic activity of these compounds with their progestational activities. 17α-Vinyltestosterone (33) and 17α-ethynyltestosterone (33–36) were the first orally active progestational agents made by partial synthesis. The latter was four times as active as the former, both orally and parenterally, and parenterally it had one third the activity of progesterone (33). 17α-Ethynyl-19-nortestosterone was five times as potent as 17α-ethynyltestosterone orally (37–39). Thus, just as with anabolic activity, removal of the 19-methyl group augments the progestational activity of the molecule. This is also true of progesterone itself: 19-norprogesterone has four to eight times the potency of progesterone (39–41).

No study of structure-activity relationships achieves its purpose unless it suggests further approaches. Although the Δ^4-3-keto configuration (or structures isomerizable thereto) appears to be important for high progestational activity, it is no special prerequisite for anabolic activity. Thus, reduction of the 3-keto group of 17α-ethyl-19-nortestosterone yields 17α-ethyl-Δ^4-3β,17β-19-norandrostenediol (Fig. 7, I), an anabolic agent more active than the parent compound but also more androgenic[2] (24). A compound lacking the 17α-ethyl group, but otherwise identical, *viz*, Δ^4-3β,17β-19-norandrostenediol (Fig. 7, II), is a still more active and long-acting anabolic agent, but is much more androgenic[3] (24). The high activity of these reduction products of the 19-nor series suggests assay of the corresponding derivatives of the vinyl and ethynyl compounds. The 17-alkyl side-chain confers oral activity and would appear to decrease androgenicity, comparing the 17α-ethyl substituted derivative (Fig. 7, I) with Δ^4-3β,17β-19-norandrostenediol itself (Fig. 7, II).

In general, the 3α-OH compounds are more potent androgens than their β isomers (*e.g.*, androsterone versus isoandrosterone; and androstane-3α,-17β-diol (Fig. 7, IIIa) versus androstane-3β,17β-diol, the former being a clinically effective anabolic agent and several times more potent an androgen than the latter (42)). Thus, one might predict that, of the isomeric reduction products, the 3α-hydroxy compounds would be even more active than the 3β-hydroxy derivatives shown (Fig. 7, I and II).

The 4,5-double bond would appear to be preferable to the 5,6-position, in view of the low activity of methylandrostenediol (Fig. 7, IV) (14, 42).

Saturated 3-keto compounds of the 19-nor series should also be evaluated because of the high physiologic activity of androstane-17β-ol-3-one (Fig. 7, V) (43). Removal of the 19-methyl group and substitution by an alkyl side-chain at position 17 should confer oral activity, reduce the androgenicity below that of the parent compound, and maintain anabolic ac-

[2] Tested as the 3-propionate.

[3] Assayed as the 3,17-dipropionate.

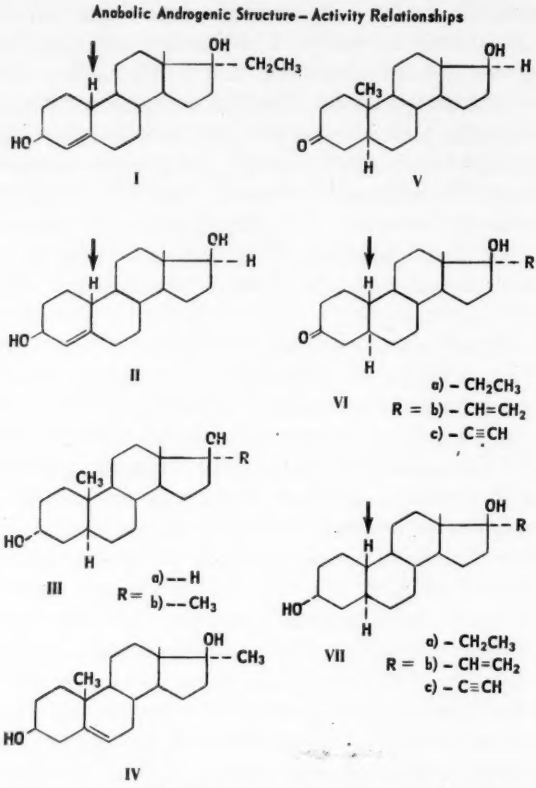

FIG. 7. Anabolic-androgenic structure-activity relationships. The potent anabolic agent 17α-ethyl-Δ^4-3β,17β-19-norandrostenediol (No. I), especially if the 3-hydroxy were in the α-configuration, is a possible metabolite of 17α-ethyl-19-nortestosterone. Similarly, the 17α-ethyl, vinyl and ethynyl derivatives of 19-norandrostane-17β-ol-3-one (No. VI) and 19-norandrostane-3α, 17β-diol (No. VII), especially the latter, are possible metabolites of the corresponding Δ^4-3-keto compounds.

tivity in the 17α-ethyl, vinyl and ethynyl derivatives of 19-norandrostane-17β-ol-3-one (Fig. 7, VI). The saturated compounds should be of the androstane rather than of the cholane series, since the latter do not ordinarily exhibit desirable physiologic properties, although androgenically inert.

The 3α-hydroxy derivatives of the 19-norandrostane series would be expected to be of the same order of activity as these 3-keto compounds. Thus, in the 19-methyl series, androstane-3α,17β-diol (Fig. 7, IIIa) and 17α-methylandrostane-3α,17β-diol (Fig. 7, IIIb) are clinically effective anabolic

agents (14, 42). Higher homologs of the 19-nor series derived from the compounds of the present study, *i.e.*, the 17α-ethyl, vinyl and ethynyl derivatives of 19-norandrostane-3α,17β-diol (Fig. 7, VIIa, b and c) would be expected to show an even more favorable ratio of anabolic activity to androgenicity.

The anabolic activity of the Δ^4-3-keto compounds we have studied could actually reside in their metabolites, produced in rather low yield from the parent compounds. Possible examples of such metabolites would be 17α-ethyl-Δ^4-3β,17β-19-norandrostenediol (Fig. 7, I), and the 17α-ethyl, vinyl and ethynyl derivatives of 19-norandrostane-17β-ol-3-one (Fig. 7, VI), and 19-norandrostane-3α,17β-diol (Fig. 7, VII).

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THYROID-ANDROGEN INTERRELATIONS AND THE HYPOCHOLESTEREMIC EFFECT OF ANDROSTERONE*

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ABSTRACT

Myxedema is associated with a profound change in the metabolism of androgen. Endogenous production of androsterone was very low in 6 myxedematous patients, and a much smaller fraction of exogenous testosterone-4-C^{14} was converted to androsterone than in euthyroid subjects. In 1 hyperthyroid patient studied the reverse was observed, in that endogenous androsterone production was elevated. Administration of triiodothyronine to myxedematous patients altered this defect in metabolism and restored an essentially normal metabolic pattern for both endogenous and exogenous androgen. Administration of triiodothyronine to euthyroid subjects produced a similar change in endogenous and exogenous androgen metabolism, in that androsterone production was materially increased. From these results the hypothesis was made that some of the peripheral manifestations of excess or deficit of thyroid hormone might be mediated by the metabolites of steroid androgens. In confirmation, it was found that androsterone caused a significant decrease of the serum cholesterol level in myxedematous patients, in subjects with hypercholesterolemia of varied origin, and in normocholesteremic subjects. One myxedematous patient showed an increase in basal oxygen consumption during treatment with androsterone.

THIS study is concerned with observations on the influence of thyroid hormone on the production and peripheral transformation of steroids, in order to inquire into the mediation of the action of thyroxine through an interplay of hormonal effects. This novel perspective of the biologic function of thyroid hormone was provided by the recent observation (1) that triiodothyronine increased the production of androsterone, a major metabolite of androgen. In contrast, a patient with untreated myxedema ex-

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hibited an extreme diminution in androsterone formation when compared with a normal subject. With continued study, it has been established 1) that myxedema is associated with a profound change in the metabolism of androgen, 2) that treatment with triiodothyronine can restore this deranged metabolism toward normal, 3) that triiodothyronine in the normal subject effects a significant deviation in androgen metabolism, and 4) that androsterone, so materially influenced by thyroid hormone, can produce one of the biologic actions normally associated with thyroid hormone itself. These findings demonstrate a "thyromimetic" action of a steroid but, in addition, there are broader implications for the larger problem of the metabolites of hormones as mediators of biologic processes. They offer a possible means of dissociation and duplication of the peripheral effects of hormones by the administration of specific metabolites.

The studies to be described fall into 3 groups: 1) the definition of the quantity and kind of endogenous androgen metabolites in myxedematous, euthyroid and hyperthyroid subjects, 2) the effect of deficiency or excess of thyroid hormone on the metabolism of exogenous androgen, and 3) investigation of the "thyromimetic" effect of androsterone.

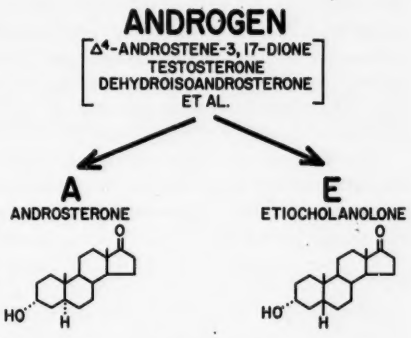

FIGURE 1

CHEMICAL ORIENTATION

Numerous investigations (2) have established that the major end-products of androgen metabolism in man are the stereoisomers, androsterone, and etiocholanolone, shown in Figure 1. These steroids are structurally identical except for the orientation of the hydrogen atom at C-5, which is α in androsterone and β in etiocholanolone. This seemingly minor chemical difference profoundly influences the biologic properties of the compounds. The precursors of these steroids are secreted by both the adrenal and testis. The 2 androgen metabolites are for the most part conjugated with glucuronic acid and are found in this form in urine; they can be readily freed from their conjugates and each isolated and measured precisely. In order to consider the relations of these 2 metabolites we have employed what is termed the "androsterone fraction." This value represents the percentage of the total androsterone plus etiocholanolone which is comprised by androsterone alone.

METHODS

For the determination of endogenous steroid production, complete 24-hour urine collections were obtained and the accuracy of the collection verified by measurement of creatinine. The analytical methods have been completely described (3, 4); briefly, they consisted of hydrolysis with β-glucuronidase[1] for five days followed by continuous extraction with ether at pH 1. The neutral fraction was separated from this extract; the acidic plus phenolic fraction was combined with the residual urine and the mixture was acidified to 1 *N* with sulfuric acid and extracted continuously with ether for forty-eight hours. A second neutral fraction was prepared from this extract. Each neutral extract was then treated with Girard's reagent T to yield the ketonic fraction. The individual steroids were isolated by paper chromatography and the quantity of each was determined by the modified Zimmermann reaction after elution from the paper.

In the studies of exogenous androgen metabolism, tracer doses of radiocarbon-labeled testosterone were employed. Approximately 1 microcurie (μc.) of radiochemically pure testosterone-4-C^{14} (specific activity 4 μc. per mg.) was dissolved in 1 ml. of absolute ethanol and this was added to approximately 150 ml. of sterile 5 per cent dextrose solution. The infusion bottle and tubing were weighed before administration and after the injection was complete. The solution was infused intravenously over a period of thirty minutes. A portion of the infusion solution was analyzed for radioactivity and the precise dose was calculated from this radioactivity and the weight of the solution administered. A small portion of the radioactive hormone was adsorbed by the polyethylene tubing connecting the bottle of solution with the needle in the vein. The tubing was extracted with ethanol and ethyl acetate and the extracts were assayed for C^{14}. This value (usually between 7 and 11 per cent of the total amount of C^{14}) was subtracted from the calculated dose in order to obtain the actual amount of testosterone-4-C^{14} administered.

The analyses for androsterone and etiocholanolone in the labeled testosterone experiments were performed by reverse isotopic dilution on individual 24-hour urine collections that had been processed by methods similar to those employed for study of the endogenous production.

Reverse isotopic dilution analysis was performed as follows. A portion of each neutral extract (10–100 per cent dependent upon the amount of radioactivity) was added to a known amount of analytically pure androsterone and etiocholanolone used as carrier. The weight of each carrier steroid was usually about 40 mg. The mixture was dissolved and chromatographed on a column of silica gel containing 40 per cent ethanol as the stationary phase and 1 per cent ethanol in a mixture of 30 per cent methylene chloride and petroleum ether as the developing solvent. The fractions from the chromatogram were examined by infrared spectrometry to establish the beginning and end of the elution of each compound. The major portion of the crystalline material so obtained was then combined and repeatedly recrystallized from acetone-petroleum ether until the specific activity was constant. Multiplication of the weight of added carrier steroid by the specific activity of the final product gave the total radioactivity initially in the neutral extract as that compound. From these values were calculated the portion of the neutral extract present as androsterone and etiocholanolone.

Radioactive samples were counted in a Tracerlab SC-50 automatic sample changer at or near the "infinitely thin" range, with appropriate correction for self absorption where necessary.

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[1] β-Glucuronidase, known as Ketodase, was obtained from the Warner-Chilcott Laboratories, a division of Warner-Lambert Pharmaceutical Company, New York, N. Y.

The clinical studies were performed on patients hospitalized in the metabolic ward of the James Ewing Hospital. Cholesterol was measured by a minor modification of the Schoenheimer and Sperry (5) method in the Department of Biochemistry of Memorial Center. The clinical status of the euthyroid and myxedema groups was established by the usual criteria, including measurement of serum protein-bound iodine concentration in most instances.

RESULTS

Endogenous androgen production and metabolism

The amounts of androsterone (A) and etiocholanolone (E) formed from endogenous androgen precursors were determined in 6 untreated myxedematous patients, 9 euthyroid control subjects, and 1 hyperthyroid patient. These data are listed in Table 1. Additional control values were reported in earlier studies (3, 6). The daily production of A plus E was approximately 1.0 mg. for the myxedema patients as compared to 4.5 mg. for the control group. Androsterone constituted 44 per cent of the total of A and E in the control subjects, but only 15 per cent in the myxedema group. Therefore, there was both an absolute and relative decrease in androsterone production by the myxedema patients. It is of incidental interest that a single hyperthyroid patient (Table 1) showed the exact reverse of the myxedematous patients, in that the level of androsterone was more than twice the level of etiocholanolone, *i.e.*, the androsterone fraction was 68 per cent.

Two patients with myxedema (SL and KA) and 3 control subjects (PD, FN and MY) were treated with triiodothyronine (T_3) by mouth in a dosage of 200 μg. per day for a minimum of ten days. There was an increase of A and E from 0.6 mg. to 1.9 mg. per day in one of the myxedema patients, and no change in the other; the "androsterone fraction" increased from 17 to 26 per cent in one patient and from 22 to 50 per cent in the other. The sum of A and E was virtually unchanged in 2 of the control subjects (FN and PD) and increased from 3.1 mg. to 4.4 mg. in the other (MY). The "androsterone fraction" increased in all subjects as a consequence of triiodothyronine administration. The effect of triiodothyronine was to produce a relative increase in conversion of endogenous androgen precursors to androsterone in all subjects and an absolute increase of both androsterone and etiocholanolone in 1 of the myxedema patients.

Exogenous androgen metabolism

The metabolism of labeled testosterone was studied in order to determine whether there was an effect of thyroid hormone on the peripheral metabolism of androgen. Table 2 lists the recoveries of administered radioactivity in the urine of the first and second days and the amount of testosterone transformed to A and E as determined by reverse isotopic dilu-

TABLE 1. ENDOGENOUS ANDROGEN PRODUCTION IN EUTHYROID, HYPERTHYROID AND MYXEDEMATOUS PATIENTS; EFFECT OF TRIIODOTHYRONINE (T_3)

Patient	Diagnosis	Treatment	Androsterone excretion (mg./24 hrs.)	Etiocholanolone excretion (mg./24 hrs.)	Androsterone fraction (%)
Name, age & sex					
FN —38 M	Multiple sclerosis	Control	2.2	3.3	40
		T_3	3.1	2.7	53
MY—41 F	Multiple sclerosis	Control	1.2	1.9	39
		T_3	2.5	1.9	57
PD —17 F	Stein-Leventhal syndrome	Control	3.8	3.9	49
		T_3	5.3	2.3	68
MN—56 M	Multiple sclerosis	Control	2.2	2.2	50
CO —69 M	Prostate cancer	Control	1.1	1.6	41
FA —73 M	Prostate cancer	Control	1.0	1.7	37
BW—34 M	Normal	Control	3.4	3.6	49
FS —34 M	Xanthoma tuberosum	Control	1.5	3.8	28
NZ —68 M	Cancer of rectum	Control	1.2	0.9	57
SL —72 M	Myxedema	Control	0.1	0.5	17
		T_3	0.5	1.4	26
KA —34 F	Myxedema	Control	0.2	0.7	22
		T_3	0.4	0.4	50
RS —66 M	Myxedema	Control	0.3	1.2	20
KP —57 F	Myxedema	Control	0.2	1.4	12
WR—67 M	Myxedema	Control	0.1	0.9	10
SA —60 M	Myxedema	Control	0.1	0.8	11
KP —10 M	Myxedema	Control	*	*	
DS —47 F	Hyperthyroidism	Control	3.2	1.5	68

* Values too low to permit accurate determination.

tion. The total excretion of radioactivity derived from testosterone during the first forty-eight hours was 70 per cent in the control group and 60 per cent in the myxedema patients. Only negligible amounts of radioactivity were excreted on subsequent days. The recovery of urinary radioactivity

TABLE 2. TESTOSTERONE-4-C^{14} METABOLISM IN EUTHYROID, HYPERTHYROID AND MYXEDEMATOUS PATIENTS; EFFECT OF TRIIODOTHYRONINE (T_3)

Patient & age	Treatment	Urinary radioactivity*		Androsterone†	Eiocholanolone†	Androsterone fraction (%)
		Day 1	Day 2			
Euthyroid						
FN —38	Control	50	8	36	44	45
	T_3	64	13	52	27	66
MY—41	Control	61	6	26	46	36
	T_3	64	4	57	27	68
MN—56	Control	70	8	43	38	53‡
CO —69	Control	54	6	53	41	56‡
FA —73	Control	60	7	19	54	26
BW—34	Control	57	7	49	35	58‡
FS —34	Control	80	6	24	42	36
Myxedema						
SL —72	Control	49	14	6	51	11
	T_3	44	8	14	41	25
RS —66	Control	58	8	10	60	14
WR—67	Control	50	7	8	68	11
KP —10	Control	59	0	8	49	14‡
SA —60	Control	54	11	2	14	12
Hyperthyroid						
DS —47	Control	51	4	57	13	82‡

* Per cent of administered dose per 24 hours.

† Per cent radioactivity in neutral steroid fraction of Day 1 plus Day 2.

‡ Day 1 values only.

in the neutral steroid fraction following the 2 hydrolytic procedures was excellent (85 to 98 per cent) in all except 2 instances in which only 56 and 73 per cent of the urinary radioactivity was recovered (KP and SA). No explanation can be advanced for these 2 discrepancies. There was a small difference between the myxedematous patients and the control subjects in the total amount of radioactivity in androsterone and etiocholanolone after administration of testosterone-4-C^{14}. Thus, in the hypothyroid patients (excepting SA) 55 to 76 per cent of the total neutral urinary radioactivity was found as these 2 compounds, whereas in the control series androsterone and etiocholanolone together accounted for from 66 to 94 per cent of the neutral urinary radioactivity, with only 1 subject showing a value below 70 per cent. One of the myxedematous patients (SA) had only 16 per cent of the neutral radioactivity as the 2 metabolites examined, with the remainder of the radioactivity in the form of as yet unrecognized steroids.

Table 2 lists the percentages of the total neutral steroid measured as androsterone and etiocholanolone for the first and second days. A striking difference is immediately evident between the 2 groups of patients. For myxedematous subjects, androsterone constituted from 2 to 10 per cent of the neutral steroids, and for the control group, from 19 to 53 per cent. The single hyperthyroid patient showed 57 per cent transformation to androsterone.

One myxedematous patient, who was made euthyroid by treatment with triiodothyronine, showed an increase in endogenous androgen production and the expected alteration in metabolism. There was a clear change in the metabolism of exogenous testosterone-4-C^{14} in this patient, since the production of androsterone more than doubled when he was euthyroid as contrasted with his hypothyroid period. Simultaneously, etiocholanolone production changed, albeit to a smaller extent, in that 55 per cent of the tracer was metabolized to this end-product when the patient was myxedematous as compared with 41 per cent when he was euthyroid.

The biologic activity of androsterone

Because of these correlations it appeared possible that androsterone might mediate some of the biologic activity ascribed to thyroid hormone. To examine this hypothesis the effect of androsterone on serum cholesterol concentration was studied in 4 patients with myxedema, 7 patients with hypercholesteremia, and 5 normocholesterolemic patients with various other disorders. The steroid, in a single daily dose of 50 mg., was given intramuscularly in sesame oil containing 15 per cent benzyl alcohol, for periods of from three to thirty-four days. The pertinent data are listed in Table 3.

TABLE 3. EFFECT OF ANDROSTERONE ON SERUM CHOLESTEROL LEVEL (MG./100 ML.)

Patient	Diagnosis	Serum cholesterol		
Name, age & sex		Control*	During admin. of androsterone†	% Change
R—66 M	Myxedema	268	161	−40
S —60 M	Myxedema; ASHD;‡ diabetes mellitus	377	282	−25
W—66 M	Myxedema; gout	221	133	−40
L —77 F	Myxedema; ASHD	321	262	−18
A —14 F	Nephrosis	363	242	−33
F —33 M	Xanthoma tuberosum	430	363	−16
H—57 F	Diabetes mellitus	450	358	−20
H—37 F	Hypercholesteremia; gout; virilizing syndrome	379	379	0
N—55 F	Hypercholesteremia	312	217	−27
W—57 M	ASHD	263	211	−20
D—55 M	Hypercholesteremia; dystrophia myotonica	392	290	−26
M—56 M	Multiple sclerosis	254	226	−11
S —75 F	Breast cancer; arteriosclerosis	318	193	−11
E —63 F	Rheumatoid arthritis	190	148	−22
E —53 F	Breast cancer; scleroderma	190	190	0
M—42 F	Multiple sclerosis	194	159	−18

* Average of at least 3 and up to 25 determinations over a period of 5 to 88 days, except in A—14 and N—55, for whom there was only a single measurement.

† If the serum cholesterol level decreased steadily and progressively, the lowest value is recorded; if the level declined but the lower values fluctuated, an average of these lower values is recorded.

‡ Arteriosclerotic heart disease.

There was a significant fall ($t=6.5$; $P<0.01$) in the serum cholesterol level in all 3 groups of patients. The effect was most striking and consistent in the patients with myxedema, in whom androsterone caused an 18 to 40 per cent decrease in the elevated serum cholesterol level during three to twenty-three days of treatment. The decrease was evident in both free and esterified cholesterol fractions. Following cessation of androsterone, the serum cholesterol concentration returned to pretreatment levels in all but 2 of the patients studied. The 2 exceptions were myxedematous subjects in whom the serum cholesterol remained at the level attained during administration of androsterone. Several other steroids were administered similarly in the same vehicle and with the patients under the same dietary

regimen, without influence upon the serum cholesterol level.

It was possible to follow the basal oxygen consumption accurately in only 1 of the 4 myxedematous patients. The pretreatment level was 85 ± 7 cc. per square meter per minute, and during administration of androsterone the value was 99 ± 6 cc. per square meter per minute. Four of 11 patients in whom adequate studies were made showed a slight but significant ($t = 3.6$; $P = 0.05$) fall in the serum uric acid level during androsterone treatment.

In about one third of the patients treated, some local tenderness and induration developed at the injection site and in about one quarter there was a transitory fever up to 102° F. These reactions required discontinuation of the steroid in some patients, largely due to local discomfort. There was no correlation between these effects and the fall in serum cholesterol concentration.

DISCUSSION

Effect of thyroid on androgen production

It has been demonstrated that the level of thyroid function profoundly influences the production of endogenous androgen and the stereochemistry of the metabolites derived from both endogenous and exogenous hormone. Hypothyroidism was uniformly characterized by an absolute and relative *decrease* in the production of androsterone. In contrast, the administration of triiodothyronine to either euthyroid or hypothyroid patients produced both an absolute and relative *increase* in the production of androsterone. Hyperthyroidism, in the 1 case studied, was associated with a high endogenous "androsterone fraction." Since this thyroid effect was also demonstrated on the metabolism of synthetic exogenous testosterone, the site of its action on the specific stereochemical transformation is in the peripheral tissues and not at the level of glandular production. There can be no question that the findings indicate an increase in the actual physiologic production of androsterone since, in the steady state, urinary excretion is equivalent to production. In addition, the absence of any renal excretory factor in this effect is clearly indicated by the finding that there was no appreciable difference between the control and hypothyroid groups either in the recovery of urinary radioactivity derived from testosterone or in the total excretion of the 2 metabolites, androsterone and etiocholanolone.

The low total production of androgenic hormones in myxedema represents another type of effect of thyroid deficiency on steroid metabolism. This reduction of the glandular production of the precursor androgenic hormone is distinct from the other peripheral effect of thyroid which results in an alteration in the subsequent metabolism of androgen. In this

connection one may compute the daily production of androgen in both myxedematous and control subjects in the manner earlier employed by Fukushima and co-workers (7). When this is done there is again a clear distinction between the myxedematous subjects, whose daily production was estimated to be between 2 and 3 mg. per day, and the control series with an estimated androgen production of between 5 and 14 mg. per day. It is evident that thyroid hormone influences the quantity of androgen production as well as the metabolic pattern of the hormone after its secretion by the glands. The diminished production of steroids in myxedema as well as the slow response to treatment has been noted in previous measurements of total urinary 17-ketosteroids (8, 9). This latter determination, which provides only a nonspecific measure of the chromogenic properties of both steroid and nonsteroid materials in a urinary extract, is ill-suited to the disclosure of specific abnormalities of metabolism, and for this reason the characteristic defects found in these studies were hitherto undetected.

Myxedematous Subject SL had an increase in total androgen production following triiodothyronine treatment, to a level consistent with that observed in euthyroid subjects of his age group. This result, in a sense, completes the demonstration of the influence of thyroid hormone on androgen metabolism in that it was shown that the observed abnormality was transformed in the expected direction even in this patient with long-standing myxedema.

Pathways of hormone metabolism

Since much of the discussion of these results revolves about the relative proportion of androsterone and etiocholanolone, *i.e.*, the "androsterone fraction" derived from precursor hormones, it is pertinent to examine the knowledge of this relationship. In 2 studies from these laboratories published about five years ago (7, 10) it was shown that the metabolic transformation of injected testosterone closely paralleled the production of androsterone and etiocholanolone from endogenous sources measured simultaneously or at different times. There appeared to be little difference between the metabolism of small (10 mg.) and large (100 mg.) amounts of exogenous testosterone, and only a minor difference in 1 subject between the values after intramuscular and intravenous routes of administration. It was evident from these prior studies that there was variation in the "androsterone fraction" and this provided a metabolic pattern characteristic of the individual subject. Age *per se* is not the principal determinant of the stereochemistry of androgen metabolism, a conclusion tentatively drawn from the earlier data. The studies reported in the present series present impressive evidence in support of these conclusions. Considering all of the subjects in Tables 1 and 2, it is clear that there were appreciable

differences between individuals. There was, however, good agreement between the metabolism of endogenous hormonal precursors to androsterone and etiocholanolone and the transformation of exogenous testosterone to these 2 metabolites. This close correlation clearly implies either identity or close structural similarity of the precursors.

The present studies *in vivo* and with human subjects disclose a group of stereospecific chemical reactions utilizing known substrates in which thyroid hormone acts as an essential mediator. They confirm and extend the conclusions reached in our preliminary report. It has been shown by McGuire (11) with rat liver mitochondria that thyroxine considerably increased the amount or activity of an enzyme system that reduced cortisone to "allodihydrocortisone." Apart from the species difference, this steroid reductase is almost surely distinct from the enzyme systems that transform testosterone, yet there is an obvious parallel in both the stereochemistry of the products and the fact that these thyroid-influenced enzyme systems have steroids as their substrates.

The fact that these substrates for thyroid hormone activity are also hormones themselves reveals a new pathway for hormone action, particularly with respect to the ultimate production of a biologic effect as a result of the interplay of several hormones. By this mechanism, a hormone can influence the enzymes controlling the metabolism of another hormone and thereby produce profound effects in the biologic systems normally under the control of the second hormone.

The "thyromimetic" effect of androsterone

The changes in androsterone seen in relation to the level of thyroid function suggested the possibility that some of the recognizable effects of thyroid deficiency or excess might be mediated by the availability of androsterone. In myxedema, apart from the clinical signs, the most evident chemical findings are the low oxygen consumption and the elevated serum cholesterol level. It has been shown that androsterone decreased the serum cholesterol level in all the myxedema patients, and it appeared that oxygen consumption increased in the 1 patient studied. The hypocholesteremic effect was not limited to myxedema but was manifest in patients with hypercholesteremia as well as subjects in whom cholesterol was initially at a normal level. This demonstration indicates that androsterone shares at least one property with thyroid hormone.

Androsterone may be the prototype of a new class of substances with potential advantage for the treatment of hypercholesteremia. This is noteworthy, since the androgens from which androsterone is derived have often been associated with hyperlipemic states, especially because of the increased incidence of atherosclerosis in males. Since androsterone produc-

tion normally declines with age (6, 12) and in view of the influence of this metabolite on the cholesterol level as shown in this study, it seems reasonable to suggest that these events may be causally associated. Androsterone is not the sole factor concerned with serum cholesterol but, nevertheless, a deficiency of this substance may well be one of the mechanisms involved in the pathogenesis of atherosclerosis.

The physiologic role of metabolites of hormones

The "thyromimetic" activity of androsterone provides a rationale for the deliberate dissociation of the activities of a hormone by the use of metabolites of that hormone. These metabolites have been generally regarded as inactive end-products of a spent hormone. The essential problem raised by these experiments is whether the metabolites of hormones have independent physiologic function similar to, or different from the glandular secretory products from which they are derived. Triiodothyronine, a metabolite that can be formed in peripheral tissues (13), exercises many, if not all, the functions ascribed to thyroxine. With respect to steroids, we have recently demonstrated that etiocholanolone is pyrogenic (14, 15). This hitherto unsuspected property, not shared with its hormonal precursors, may have physiologic pertinence, as indicated by the finding of Bondy and associates (16) that periodicity in a disturbance of metabolism of etiocholanolone could be associated with the presence of fever in periodic disease.

The significance of the "thyromimetic" activity of androsterone goes beyond duplication and dissociation of the peripheral activities of thyroid hormone. It is a clear example of specific correction of a defect in the intermediary peripheral metabolism of a hormone by administration of the metabolite produced in inadequate amount. The net result was to effect at least partial reversal of the hypercholesteremia of myxedema, a biochemical lesion produced by the absence of thyroxine, an unrelated hormone.

The existence of this "thyromimetic" activity was inferred from the alteration in the pattern of steroid metabolism produced by thyroid hormone. The necessity for exploration of the patterns of hormone metabolism in other disease states by analytic methods of sufficient precision, such as are now available for steroids, is clearly warranted from this result alone.

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ALTERATIONS OF THYROID FUNCTION IN SEVERE HEART DISEASE, AS MEASURED BY I^{131} TECHNIQUES

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ABSTRACT

A study of 18 patients with severe heart disease following administration of an I^{131} tracer showed the following alterations in thyroid function compared with euthyroid controls. 1) a decrease in the 24-hour and 72-hour plasma levels of PBI^{131}, 2) a decrease in the 24-hour conversion ratio, and 3) a pronounced increase in salivary I^{131} activity at twenty-four hours. The thyroidal uptake of I^{131} remained the same as in euthyroid subjects. Data on hypothyroid and hyperthyroid patients are listed for comparison.

INTRODUCTION

IT HAS been found by Meschan and coworkers (1) that clearance rate factors for I^{131} tend to be lower in cardiac patients than in the population at large.

This investigation reports the results of measuring the 24-hour thyroidal I^{131} uptake, conversion ratio, I^{131} activity in saliva, and the 24-hour and 72-hour levels of plasma protein-bound iodine $(PBI)^{131}$ and inorganic I^{131}.

All patients were evaluated clinically, and classified according to clinical and laboratory data.

METHODS

A capsule containing approximately 50 μc. of I^{131} was given orally and twenty-four hours later the thyroidal uptake was measured. Blood was drawn into a heparinized syringe. After centrifuging, a 2-cc. sample of plasma was pipetted into a test tube and the radioactivity was measured in a well counter. It was then poured over an ion exchange resin column, to remove the $iodide^{131}$ in solution and permit the protein-bound iodine material to drip through (2–4). The protein-bound iodine fraction was collected underneath the column and measured in the well counter. The inorganic plasma I^{131} concentration was determined by subtracting the protein-bound $iodine^{131}$ from the total $plasma^{131}$.

The conversion ratio is the PBI^{131} activity expressed as a per cent of the total plasma I^{131} activity.

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TABLE 1. AVERAGE AND RANGE OF THYROIDAL I^{131} UPTAKE AND PLASMA DATA IN PATIENTS WITH SEVERE HEART DISEASE AND IN THOSE WITH VARIOUS THYROID DISEASES

Diagnosis	No. of patients	Thyroidal I^{131} uptake				Plasma inorganic I^{131} (% D/L)				Plasma PBI^{131} (% D/L)				Conv. ratio (%)	
		24 hrs.		72 hrs.		24 hrs.		72 hrs.		24 hrs.		72 hrs.		24 hrs.	
		Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range
Euthyroid	16	35.4	22.0–54.2	34.5	19.8–52.5	.197	.108–.320	.010	0–.055	.047	.014–.104	.088	.017–.180	19.9	8.5 – 44.0
Severe heart disease	18	35.6	17.4–65.0	36.9	12.0–66.8	.510	.111–1.91	.113	.002–.88	.032	.005–.090	.060	0–.190	8.6	.80–27.5
Primary hypothyroidism	8	17.1	10.1–29.2	—	—	.765	.213–1.64	.140	.021–.30	.042	.014–.087	.046	.032–.060	6.2	2.9 – 9.1
Hyperthyroidism	14	74.5	46.3–94.0	64.0	34.0–80.7	.077	0–.336	.120	0–.336	.802	.076–1.72	1.36	.190–2.50	89.9	57.2 – 100

Saliva was collected at twenty-four hours without any secretogogue and measured in the well counter.

All radioactivities are expressed as percentage of the dose per liter (per cent D/L).

RESULTS

Table 1 shows the averages and ranges of thyroidal I^{131} uptake and plasma values in 16 euthyroid subjects, 18 patients with severe heart disease, 8 with primary hypothyroidism and 14 with hyperthyroidism. The I^{131} uptake in the cardiac patients was the same as in the euthyroid subjects at both twenty-four and seventy-two hours.

Plasma PBI^{131} values in the cardiac patients were moderately but significantly below those in the euthyroid subjects at both twenty-four and seventy-two hours. At twenty-four hours the average value for PBI^{131} was 0.032 per cent D/L in the cardiac group and 0.047 per cent D/L in the euthyroid group; at seventy-two hours the respective values were 0.060 and 0.088 per cent D/L. These data were analyzed statistically by the *t* test and found significant to more than the 90 per cent level.

TABLE 2. AVERAGE AND RANGE OF SALIVARY I^{131} ACTIVITY IN PATIENTS WITH SEVERE HEART DISEASE AND IN THOSE WITH VARIOUS THYROID DISEASES

Diagnosis	No. of patients	Salivary I^{131} (% D/L)	
		24 hrs.	
		Average	Range
Euthyroid	16	8.8	5.6 –14.2
Severe heart disease	18	26.9	5.82–81.5
Primary hypothyroidism	8	31.6	14.8–47.0
Hyperthyroidism	14	1.54	0.66– 4.8

The conversion ratio in the patients with severe heart disease (8.6 per cent) was much less than in the euthyroid subjects (19.9 per cent) and approached the values found in hypothyroidism (6.2 per cent).

In Table 2 are listed the averages and ranges of salivary radioactivity at twenty-four hours. In the cardiac patients the salivary radioactivity was 26.9 per cent D/L; in the euthyroid subjects, 8.8 per cent D/L; in the hypothyroid patients, 31.6 per cent D/L; and in the hyperthyroid patients, 1.54 per cent D/L (5–12).

DISCUSSION

Thus it is seen that in cardiac patients following administration of I^{131}, although the thyroidal uptake remains the same as for the euthyroid group, the plasma PBI^{131} level and the conversion ratio are lower, but the plasma inorganic I^{131} level and the salivary activity are higher.

The decrease in PBI^{131} would seem to indicate that thyroid hormone synthesis has been impaired, and the decrease in the conversion ratio reflects both the decrease in plasma PBI^{131} and the increase in inorganic I^{131} content. The increase in salivary radioactivity does not appear to be merely a reflection of the increase in the inorganic iodine level of the plasma, for the ratio between salivary activity and the plasma inorganic iodine activity is not constant but varies with the disease state (12).

If the salivary activity is used as an index of hypothyroidism, one should be cognizant that the average and range in cardiac patients are similar to those in patients with primary hypothyroidism.

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DISTRIBUTION OF SERUM RADIOIODINATED COMPOUNDS IN EUTHYROID AND HYPERTHYROID PATIENTS FOLLOWING HYPOPHYSECTOMY*

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ABSTRACT

Hypophysectomy performed in 6 euthyroid patients for the treatment of cancer was followed by a changed pattern of distribution of radioiodinated compounds in the serum (after a tracer dose of I^{131}). Radioactivity was present almost entirely in the iodide fraction, with virtually none in the thyronine fraction. In contrast, hypophysectomy performed in 2 hyperthyroid patients (1 of whom had ophthalmopathy) and 2 euthyroid patients with the ophthalmopathy of Graves' disease was followed by a normal or hyperthyroid pattern of distribution of the iodinated compounds in the serum.

INFORMATION about the effect of hypophysectomy upon the distribution of iodine in serum has heretofore been based only on the results of studies in animals (1). In this investigation, advantage has been taken of the clinical use of hypophysectomy, to extend these observations to man. Euthyroid patients undergoing surgical removal of the pituitary for the treatment of cancer, and hyperthyroid and euthyroid patients with the ophthalmopathy of Graves' disease similarly operated upon, were studied. In the euthyroid cancer patients there were marked changes postoperatively in the distribution of serum iodinated compounds, comparable to those seen in the rat. Surprisingly, however, little if any change in distribution was observed postoperatively in the patients with Graves' disease.

Studies in man on the effect of hypophysectomy on thyroid function (2) have revealed a low to low-normal thyroidal uptake of I^{131} within one to two months after operation and a decrease in the level of serum protein-bound iodine (PBI). Studies in the rat have added information concerning changes in the distribution of iodine, both in the thyroid and in the serum

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(1). In the serum, a pronounced decrease in the I^{131}-labeled thyronine (T_4+T_3) fraction and a relative increase in iodide^{131} were noted. In the thyroid, marked reductions in the total uptake of I^{131}, in the iodide-concentrating capacity, and in the rates of synthesis of labeled monoiodotyrosine (MIT), diiodotyrosine (DIT) and thyroxine (T_4) were found, the labeled T_4 content being reduced to a much greater extent than that of the labeled iodotyrosines. A decreased rate of release of labeled T_4 was also observed.

METHODS

Hypophysectomy was performed surgically upon 2 actively hyperthyroid patients (1 with ophthalmopathy, as well), 2 euthyroid patients with ophthalmopathy, and 6 euthyroid patients (1 with prostatic and 5 with mammary cancer). The study was undertaken to elaborate further the clinical observation (made by D.V.B.) that the thyroidal I^{131} uptake and the serum PBI level in the 2 actively hyperthyroid patients failed to drop precipitously following hypophysectomy. The clinical details of these patients are being presented elsewhere (3). These, and the euthyroid patients with the ophthalmopathy of Graves' disease, were operated upon by Dr. Bronson Ray of the New York Hospital-Cornell Medical Center. The cancer patients were operated upon at the Frances Delafield Hospital by Dr. William Bridges. A labeling dose of 100 to 300 μc. of I^{131} was given and blood samples drawn thereafter, either at seventy-two hours or serially.

Chromatography was performed by the method of Block, Mandl and Werner (4).

Most patients were studied within one to three months after hypophysectomy. Data are available on 1 cancer patient and 1 hyperthyroid patient, one year postoperatively. The other thyrotoxic patient and 1 euthyroid patient with ophthalmopathy were studied both before and after hypophysectomy. Replacement therapy in the 2 groups, when provided, was similar.

RESULTS

In Table 1 are listed the percentage compositions of the labeled iodinated compounds in the serum of the hyperthyroid and the cancer patients. Results of chromatography with the two solvent systems employed were virtually identical.

After hypophysectomy in the euthyroid patients, the combined labeled T_4 and T_3 fractions constituted between a trace and 11 per cent of the total radioactivity three days after labeling, whereas iodide^{131} constituted between 77 per cent and 99 per cent (Table 1). In 1 hyperthyroid patient (H.G.) the relative values of labeled T_4 and T_3 combined and of iodide three days after labeling were 18 per cent and 82 per cent respectively, at three months postoperatively, but 70 per cent and 30 per cent respectively at six months, and 33 per cent and 67 per cent respectively at one year after hypophysectomy. In the other hyperthyroid patient, the thyronine fraction contained 85 per cent of the activity as late as one and a half months after hypophysectomy—about the same value as before operation. Practically no change from the normal distribution occurred following operation in the

TABLE 1. DISTRIBUTION OF I^{131} IN THE NONPROTEIN FRACTION OF SERUM IN HYPERTHYROID AND IN CANCER PATIENTS FOLLOWING HYPOPHYSECTOMY

Patient	Diagnosis	Mos. after hypophysectomy	Days after I^{131}	Serum PBI^{131} (%/liter)	Relative per cent I^{131} as iodocompound			
					DIT	MIT	I^-	T_4 & T_3
S.K.	Ca. prostate	1½	3		7	9	77	7
C.H.	Ca. breast	3	3		<1	<1	89	11
I.W.	Ca. breast	2	3		<1	<1	94	6
F.M.	Ca. breast	12	3		<1	0	99	1
E.M.	Ca. breast	3	3		<1	<1	99	1
			5		<1	<1	99	1
			10		activity too low			
H.G.*	Toxic diff. goiter with ophthalmopathy	3	3	0.25	<1	<1	82	18
		6	3		<1	<1	30	70
		12	3	0.6	<1	<1	67	33
			5		<1	<1	34	65
S.M.*	Toxic diff. goiter	before op.	3	2.3	<1	<1	5	95
		½	3	1.5	<1	<1	1	99
		1½	3		<1	<1	15	85
G.J.†	Euthyroid, with pretibial myxedema and noninfiltr. ophthalmopathy	2	2		0	0	96	4
		2¾	2		<1	2	92	6
			3	0.1	<1	<1	56	44
			6		<1	<1	21	79
M.R.†	Euthyroid, with infiltr. ophthalmopathy	before op.	4		<1	<1	32	68
		½	3	0.35	<1	<1	15	85
		2	3		0	0	55	45
H.A.‡	Normal; no operation		1		<1	<1	96	4
			3		<1	<1	51	49
			7		<1	<1	14	85

* Thyroidal 24-hour I^{131} uptakes greater than normal.
† Thyroidal 24-hour I^{131} uptakes <10%.
‡ From Werner and Block (10).

2 euthyroid ophthalmopathic patients. The effect of the length of time after labeling upon the distribution of iodine was observed in each group (Table 1).

DISCUSSION

The persistently normal or hyperthyroid distribution of the labeled iodinated compounds of serum in the patients with Graves' disease undergoing

hypophysectomy was in sharp contrast to the marked alterations in distribution occurring in the prostatic and mammary cancer patients undergoing the same procedure. An immediate question concerns the completeness of hypophysectomy. It is possible that the procedure was more complete in the cancer patients than in the others, but this view is rendered less likely by the consistency of the chromatographic findings within each group. Also, extensive previous experience with the surgical procedure has shown a very high rate of success (5), although some viable pituitary cells unquestionably survive. In all patients of the present series, the clinical picture of hypopituitarism developed postoperatively.

If, then, hypophysectomy can be considered similarly complete in both groups, the persistently normal or high level of labeling of thyronines in the cases of Graves' disease suggests that the thyroid remained in a state of hyperfunction even though there was little or no hypophyseal tissue. This is consistent with the fact that the pituitary-thyroid relationship is abnormal in hyperthyroidism (6), and with the fact that hyperthyroidism can occur in patients having a greatly reduced mass of functioning anterior pituitary tissue (7, 8). It is possible that the cells of the pituitary were hyperactive before hypophysectomy in the hyperthyroid group, and that the few cells presumably remaining afterwards were thus sufficient to keep the thyroid activated, in contrast to the less active cells presumably present in the euthyroid group. It is also possible, though not probable, that by a sampling peculiarity, the Graves' disease group represented only those rare patients who maintain a persistently normal thyroidal I^{131} uptake despite a fullblown picture of panhypopituitarism (9).

The progressive increase with time in the relative amounts of thyronines in the Graves' disease group follows the pattern recently demonstrated in patients with normal thyroid function (10). The lack of this trend in the hypophysectomized patients with cancer probably reflects only the extreme slowness of the turnover rate in this group.

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THE CELLS OF THE HUMAN ADENOHYPOPHYSIS IN THYROID DISORDERS*

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ABSTRACT

Cell counts were made on pituitaries stained with the iron-PAS technique from 14 cases of myxedema and 6 cases of hyperthyroidism. In hypothyroidism there was a significant increase in the proportion of gamma cells, and a significant decrease in the proportion of alpha cells. Gamma cells were characterized by the inclusion of a large number of PAS-positive vesicles, which were also found in alpha cells, chromophobes and interstitial spaces. In hyperthyroidism there was no consistent cellular pattern. In comparing the cell counts in myxedema and hyperthyroidism, the only significant difference was the greater percentage of gamma cells in the hypothyroid group. The suggestion is made that beta cells and vesiculated gamma cells form thyrotropin, and that there is a definite difference between the pituitaries in hypothyroidism and hyperthyroidism.

INTRODUCTION

THE iron-periodic acid Schiff (iron-PAS) technique permits the clear differentiation of 5 cell types in the human adenohypophysis: alpha, beta, delta, gamma and chromophobe. In previous reports we recorded cell counts both in "normal" pituitaries from patients without endocrine disease (1) and in pituitaries from patients with adrenocortical disorders (2). The present investigation deals with cell counts in a series of pituitaries from patients with thyroid disease. These studies provided us with information about the behavior of the various cell types in endocrine disease, and allowed us to relate these cells to those described by other investigators.

METHODS

The material consisted of pituitary glands from 14 cases of untreated myxedema and 6 cases of untreated hyperthyroidism. Most of these were selected from the routine

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necropsy cases at the Toronto General Hospital; some specimens were obtained from other hospitals.

The pituitaries were stained by the iron-PAS technique, and differential cell counts were performed according to the method previously described (1). The relative proportions of the various cell types were expressed as percentages of the total number of cells. In our first study, cell counts were made in a series of patients who died of nonendocrine illnesses of various durations (1). Composite averages of the cell counts from all these cases were used as controls for all types except delta cells. The percentage of delta cells in the pituitaries of nonendocrine cases varied inversely with the duration of the final illness (1). Hyperthyroidism and myxedema were considered to be chronic diseases, even though some of the patients had additional terminal illnesses of short duration.

TABLE 1. THE EFFECT OF THYROID DISORDERS ON THE PROPORTION OF PITUITARY CELL TYPES

Endocrine condition	No. of cases	Proportion of cell types in pituitary				
		Alpha (%)	Beta (%)	Delta (%)	Gamma (%)	Chromophobe (%)
Nonendocrine control*	71	50.31±2.6†	13.01±1.4	(a) 8.46±0.8 (b) 4.56±0.7 (c) 1.93±0.4	8.33±0.6	23.04±2.4
Hypothyroidism	14	39.20±2.0	13.55±1.9	1.18±0.4	17.80±2.3	28.21±2.5
Hyperthyroidism	6	35.56±6.4	15.21±2.8	7.18±3.0	6.98±3.9	35.26±6.7
Statistical analysis (P values less than 0.05)						
Hypothyroidism: Normal		.001	—	—	.01	—
Hyperthyroidism: Normal		—	—	—	—	—
Hyperthyroidism: Hypothyroidism		—	—	—	.05	—

* The percentages of alpha, beta and gamma cells and chromophobes are the composite averages in the 3 groups of patients from our previous study who died of nonendocrine illnesses of various durations (1). These were used as controls for the present investigation. The percentages of delta cells are listed separately for the 3 groups: (a) sudden death (less than 24 hours); (b) short illness (1–14 days); and (c) long illness (more than 2 weeks). Only the long-illness group (c) was used as a control for the delta cells of the present series, since endocrine illnesses were considered to be of long duration.

† Standard Error.

RESULTS

Hypothyroidism

Cell counts were performed on the pituitaries of 14 patients who were clinically hypothyroid, and in whom little or no thyroid tissue was found at autopsy (Table 1). In comparison with the composite control group, the proportions of beta cells and chromophobes remained unchanged, but there was a significant decrease in the percentage of alpha cells, and a significant increase in the relative number of gamma cells. The proportion of delta cells was generally low, as expected in a chronic illness (Table 1).

In most cases, the presence of many gamma cells studded with magenta-colored vesicles gave the gland an unusual appearance even under low

power (Fig. 1). A few of these vesicles, which were not evident after the conventional Mallory-type staining, were seen in normal adult glands stained by the iron-PAS technique. They were much more numerous in the pituitaries from cases of hypothyroidism (Table 2). Vesicles were often found in the extracellular spaces, occasionally in alpha cells and chromophobes, but never in delta cells. No vesicles could be identified in the beta cells, but they may have been obscured by the heavy granulation. Vesiculated cells of all types were counted in 15 unselected glands from nonendo-

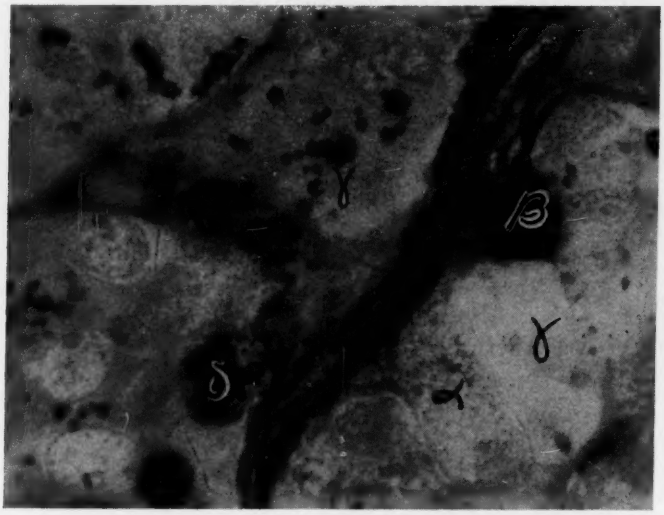

FIG. 1. Microphotograph of anterior pituitary in a patient with myxedema who died suddenly from a myocardial infarction (P-68-58, male, aged 55; Table 2). The vesicles characteristic of myxedema are located mainly in gamma cells. Alpha, beta and delta cells are also seen in this field. (Iron-PAS stain ×1350.)

crine cases, and the average incidence was found to be 1.8 ± 0.23 per cent. In contrast, the average percentage of vesiculated cells in the pituitaries from cases of myxedema was 11.7 ± 1.6 (Table 2). To determine whether the vesicles were the result of a nonspecific tissue reaction to hypothyroidism, sections from the heart, liver, kidney and pancreas in these cases were stained with the iron-PAS technique. No vesicles were found in these tissues.

The pituitaries from 2 infantile cretins[1] were also examined, but cell

[1] Specimens made available through the kindness of Dr. D. V. Hubble, Derby, England, and Dr. W. L. Donohue, Pathologist-in-Chief, Hospital for Sick Children, Toronto.

counts were not made. These glands were characterized by the virtual absence of alpha cells, and a scarcity of beta cells. As is always the case in children's glands, no delta cells were seen. Gamma cells were the predominant cell type, but no vesicles were present in these glands.

Hyperthyroidism

Cell counts were performed on the pituitaries of 6 patients who died with uncontrolled hyperthyroidism (Table 3). Four of these patients had Graves'

TABLE 2. THE ADENOHYPOPHYSIS IN HYPOTHYROIDISM

Patient		Terminal illness*	Proportion of cell types in pituitary					Vesiculated cells†
Autopsy No.	Sex and age		Alpha (%)	Beta (%)	Delta (%)	Gamma (%)	Chromophobe (%)	Per cent of total
P.68/58	M 55	Myocardial infarction	48.3	6.9	3.1	35.8	7.3	21.2
A.374/53	F 75	Myocardial infarction	28.0	16.2	0.4	28.1	27.2	16.0
A.373/55	F 64	Myocardial infarction	30.4	12.2	2.1	21.4	34.0	18.0
A.292/56	F 77	Myocardial infarction	44.4	9.6	0.7	6.5	38.9	9.1
A.496/56	M 76	Acute cholecystitis	53.6	1.3	0.6	18.8	25.6	7.4
A.61/57	F 79	Cerebrovascular accident	47.1	13.8	0.4	6.8	31.8	10.3
P.8/56	M —	Myocardial infarction	31.9	30.6	4.6	15.4	17.4	8.6
A.371/56	F 64	Uremia from chronic pyelonephritis	31.8	7.5	2.0	13.0	45.5	10.0
A.40/57	M 71	Status epilepticus	41.0	11.5	1.8	19.7	25.6	21.8
A.1535/41	F 64	Carcinomatosis	31.8	22.9	0	13.7	31.4	6.3
A.892/39	F 73	Lymphosarcoma	58.0	16.0	0.2	22.4	23.1	15.0
A.71/57	M 72	Uremia from pyelonephritis	38.4	6.6	0.1	15.3	39.5	13.1
A.57/53	F 50	Carcinoma of thyroid (nonfunctioning)	58.0	16.0	0.2	22.4	23.1	15.0
A.60/58	F 64	Ruptured aneurysm of left ventricle	39.0	17.3	0.5	20.1	22.8	1.7
		Average (14 cases)	39.20	13.55	1.18	17.80	28.21	11.67
		±Standard Error	±2.0	±1.9	±0.4	±2.3	±2.5	±1.6

* All of these cases exhibited the clinical and laboratory features of hypothyroidism, and little or no thyroid tissue was found at autopsy.

† The vesiculated cell count includes alpha, gamma and chromophobe cells with vesicles, which were also included in the counts of their respective cell types.

disease or diffuse hyperplasia, and 2 had toxic nodular goiter. No obvious difference in the percentages of pituitary cells was observed between these two types of hyperthyroidism. In fact, the whole group of cases with hyperactive thyroids was characterized by great individual variation in the percentage of cell types in the pituitary, so that comparison with the control groups did not show any significant difference. The gamma cells were not increased, nor did they contain more than a few vesicles. In spite of wide variations, when the hyperthyroid group was compared to the hypothyroid group, the percentage of gamma cells was significantly less in the hyperthyroid series. There was a high proportion of delta cells in 3 of these cases, but the average percentage of delta cells in this group did not differ significantly from that in either the normal or the hypothyroid group.

TABLE 3. THE ADENOHYPOPHYSIS IN HYPERTHYROIDISM

Patient		Thyroid condition*	Terminal illness	Proportion of cell types in pituitary				
Autopsy No.	Sex and Age			Alpha (%)	Beta (%)	Delta (%)	Gamma (%)	Chromophobe (%)
A.970/39	F 58	Diffuse hyperplasia	Thyroidectomy; died in post-op. storm	32.6	9.9	9.0	12.4	36.1
P.117/57	M 60	Toxic nodular goiter	Bronchopneumonia	17.5	22.6	17.0	24.0	18.7
A.76/46	F 54	Diffuse hyperplasia	Thyroidectomy; died of post-op. embolism	61.2	6.5	14.2	1.0	17.5
A.1610/41	M 62	Diffuse hyperplasia	Bronchopneumonia	30.0	23.5	0.8	2.0	44.0
A.1114/40	F 53	Diffuse hyperplasia	Thyroid storm	46.0	16.5	1.0	2.5	34.1
A.219/55	F 68	Toxic nodular goiter	Cardiac failure	26.1	12.2	1.1	0.0	61.2
		Average (6 cases) ± Standard Error		35.56 ±6.4	15.21 ±2.8	7.18 ±3.0	6.98 ±3.9	35.26 ±6.7

* All these cases exhibited clinical and laboratory features of hyperthyroidism.

DISCUSSION

In our previous paper (1), it was suggested that gamma cells are a heterogeneous group of PAS-positive cells which correspond to the amphophils of Russfield (3) and to the hypertrophied lightly PAS-positive cells described by Russell (4) and by Pearse (5–7). These investigators agree that a pituitary containing a large proportion of gamma cells is an actively secreting gland. By examining pituitaries stained with the iron-PAS technique from patients in different states of hormonal balance, it was possible to recognize several types of gamma cells. In normal glands most gamma cells are large, with a vesicular nucleus and finely granular cytoplasm; some are smaller, with coarse, sparse granulation (1). An additional type of gamma cell, the Crooke-Russell cell, is found rarely in normal glands, but is seen frequently in the pituitaries from patients with Addison's disease (2). In myxedema there is an increase in the proportion of still another type of gamma cell, containing many magenta vesicles. Under normal circumstances it is likely that both adenocorticotropic hormone (ACTH) and thyrotropic hormone (TSH) arise in ordinary gamma cells, and probably also in beta cells. In Addison's disease, additional ACTH may be secreted by the Crooke-Russell gamma cells, and in myxedema additional TSH may be secreted by vesiculated gamma cells.

A large number of magenta-stained vesicles is a characteristic, but not invariable feature of the pituitary in myxedema. A few vesicles are found normally in all adult pituitaries, but not in the glands of young children (5). Occasionally many such globules are seen in the pituitary of a patient who is not hypothyroid; conversely, the gland in 1 of the cases of myxedema from the present series contained a normal number of vesicles. No

vesicles were seen in the pituitaries of cretins. However, in 2 of our cases of adult hypothyroidism a tentative diagnosis of myxedema was made before the condition of the thyroid gland was known, based upon the finding of an abnormally high proportion of vesiculated cells in the pituitary. The increased secretion of TSH that occurs in myxedema (8, 9) probably takes place in the vesiculated gamma cells. Pearse observed many PAS-positive vesicles in the pituitaries of humans, as well as in rats treated with thiouracil (5). Vesiculated cells were associated with a high content of ribonucleic acid, suggesting active protein synthesis. When Burt and Velardo (10) assayed human hypophyses for thyrotropic hormone, they found that large numbers of PAS-positive vesicles were usually associated with a high TSH content of the gland.

The concept of 2 different types of gamma cells secreting ACTH and TSH respectively is supported by the appearance of the pituitary in a patient with Addison's disease who also had myxedema.[2] In this case both Crooke-Russell cells and vesiculated gamma cells were present in increased numbers.

The pituitary cell counts in the myxedema group did not provide any information as to which cells gave rise to the TSH-producing gamma cells. It is likely that some gamma cells were derived from chromophobes, but this transformation could not account for all gamma cells, as there was no significant decrease in the percentage of chromophobes. Evidence linking the beta cells with TSH secretion in the human gland is provided by Russell's observation that the granules in the hypertrophied chromophobes (analogous to our gamma cells (1)) show a beta-type reaction with the aldehyde-fuchsin technique (4). In many animal species (rat (11–13), dog (14), bat (15), mouse (16) and frog (17)) there are indications that cells with the morphologic characteristics of the beta cells of the human gland secrete TSH. For example, in the pituitaries of thyroidectomized rats, beta cells become vacuolated ("thyroidectomy cells") (11, 12). Such changes do not occur in the pituitaries of myxedematous humans. Pearse (18) attributed the secretion of glycoprotein hormones to PAS-positive mucoid cells. Because TSH is a glycoprotein (19) it seems likely that this hormone is associated with either beta or delta cells, the two types of heavily granulated PAS-positive cells. Evidence was presented in a previous publication to suggest that the delta cells of the human gland are analogous to the gonadotropes of the rat (1). It would, therefore, appear that beta cells correspond to the thyrotropes, which they also resemble histologically. Based on the results of the present study, we suggest that beta cells and vesiculated gamma cells secrete TSH.

[2] The cell counts in this case are included among those in the series of patients with adrenocortical insufficiency (2).

The beta cell is also associated with the secretion of corticotropin, which is not a glycoprotein (2). The affinity of beta cell granules for the PAS reagent may be explained by their TSH content. Purves and associates (20) thought that Crooke's hyaline change of the beta cells associated with glucocorticoid excess may be related to an increase in the secretion of TSH. No Crooke's hyaline cells were seen in pituitaries from cases of myxedema, in which there likely was an increased production of TSH. Bakke and Lawrence (21) found an increased content of TSH in the pituitaries from 4 cases of myxedema, and a subnormal content of TSH in the pituitaries from 2 cases treated with corticosteroids. We suggest that hyalinization of the beta cells may result in a reduction of their TSH content.

It is generally accepted that alpha cells are the source of somatotropin. The pituitaries of cretins show a virtual absence of alpha cells. Clinically these children show a retardation of skeletal growth (22) which may be analogous to the cessation of growth in thyroidectomized rats; these rats also have few alpha cells in their pituitaries (23, 24). In human adults with myxedema, however, the reduction in alpha cells is not great enough to produce microsplanchnia, which is a characteristic effect of somatotropin deficiency. The decrease in alpha cells is probably a nonspecific reaction to hormonal imbalance, since alpha cells are also reduced in hyperthyroidism and Addison's disease (2).

The low percentage of delta cells in myxedema may be explained by 1) the debilitating influence of chronic hypothyroidism, or 2) a deficiency of thyroid hormone, affecting specifically the pituitary gland. The high percentage of delta cells in 3 of our cases of hyperthyroidism (Table 3) stands in marked contrast to the generally low proportion of delta cells in myxedema.

The role of the pituitary gland in hyperthyroidism is not clearly understood. Earlier investigators regarded the cellular appearance of pituitaries from cases of hyperthyroidism as indicating inactivity. Both Kraus (25, 26) and Hawking (27) observed a decrease in weight and an increase in fibrous tissue in the pituitaries from cases of Graves' disease. On the other hand, Russfield (3, 28) noted an increase in the proportion of amphophils in hyperthyroidism as well as in hypothyroidism, and suggested that this indicated pituitary overactivity in both conditions. In our cases of Graves' disease, there were few gamma cells and only a normal amount of vesiculation. In one of our 2 cases of toxic nodular goiter, there were many gamma cells, but they were not vesiculated; in the other case there were virtually no gamma cells. Bakke and Lawrence's (21) report of a very high TSH content of the hypophysis in a case of untreated hyperthyroidism suggests that pituitary hypersecretion of thyrotropin may be important in the pathogenesis of this disease. However, apart from a nonspecific de-

crease in alpha cells, we found no histologic similarity between the pituitaries in myxedema and hyperthyroidism.

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ADRENOCORTICAL HYPERPLASIA OCCURRING WITH METASTATIC CARCINOMA OF THE PROSTATE: REPORT OF A CASE EXHIBITING INCREASED URINARY ALDOSTERONE AND GLUCOCORTICOID EXCRETION*

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ABSTRACT

The case is presented of a 68-year-old male with extensively metastasizing carcinoma of the prostate, in whom severe hypokaliemic alkalosis developed. The urinary excretion of aldosterone and of glucocorticoids was greatly increased and at autopsy bilateral adrenal hyperplasia was found. Despite the elevated glucocorticoid excretion, the only clinical findings suggesting Cushing's syndrome were hyperglycemia and glycosuria. Limited metabolic studies revealed renal wasting of potassium, the constant excretion of an acid urine, and the continued ability to concentrate the urine. Review of the literature suggests that there is a causal relationship in the coexistence of adrenal hyperplasia and cancer of other organs, but it cannot be stated which disease process is primary. In the present case, the prostatic carcinoma seems definitely to have developed first. The possible role of estrogen therapy and castration in the development of adrenal hyperplasia in this case should be considered.

THE coexistence of adrenocortical hyperplasia and malignant neoplasms of other organs is common enough to suggest strongly that this association is not merely coincidental. Kovach and Kyle (1) recently reviewed 7 cases (including their own) of Cushing's syndrome associated with undifferentiated carcinoma of the lung. Several other cases have been reported (2–5). The high incidence of thymic tumors in patients with

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Cushing's syndrome has been noted in several reviews (6–8). Four cases of pancreatic carcinoma were found among 114 cases of adrenal hyperfunction by Plotz *et al.* (7). Parker and Sommers (9, 10) recently reported an increased incidence of histologic evidence of adrenal hyperplasia in patients dying of cancer as compared with noncancerous controls.

It has been noted that in cases of adrenocortical hyperplasia associated with carcinoma of the lung, profound electrolyte abnormalities develop more frequently than in cases of Cushing's syndrome, causing speculation about the possible role of aldosterone (1). Urinary excretion of this hormone has been determined in 2 cases of bronchial carcinoma associated with marked hypokaliemic alkalosis and elevated glucocorticoid excretion (3, 11); in both, it was found to be low. This report describes a patient with an extensively metastasizing prostatic carcinoma in whom hypokaliemic alkalosis developed. The urinary excretion of aldosterone was elevated, as was that of other cortical hormones, and at autopsy bilateral adrenocortical hyperplasia was found.

CASE HISTORY

W.K. (No. 071074) was a 68-year-old white male, first admitted to the Hospital of the University of Pennsylvania in September 1955, with a three-year history of increasing lower urinary-tract obstruction. The past history included an episode described as "hypertensive encephalopathy" in 1953, and episodes of tachycardia for which intermittent quinidine therapy had been prescribed. Positive findings included obstruction from a symmetrically enlarged prostate, a blood urea nitrogen level of 15 mg. per 100 ml., a fasting blood sugar level of 78 mg. per 100 ml., and blood pressure 140/90 mm. Hg. A transurethral prostatectomy was performed; histologic examination of the tissue revealed adenocarcinoma. The patient then underwent bilateral orchiectomy, and treatment with diethylstilbestrol (10 mg. daily) was begun. He was later treated for two months with chlorotrianisene (a synthetic estrogenic compound) in a dosage of 12 mg. daily. In June 1956 he was admitted to another hospital because of a constricting pain in the chest. Blood pressure was 130/70, the blood urea nitrogen level was 27 mg. per 100 ml., and the fasting blood sugar level was 90 mg. per 100 ml. An electrocardiogram showed "evidence of myocardial impairment." Chlorotrianesine was started again and given for the next fourteen months. In August 1957, he was readmitted to another hospital for evaluation of rectal bleeding. Proctoscopy revealed an extrinsic rectal mass. The concentration of blood urea nitrogen was 25 mg. per 100 ml., and of serum acid phosphatase, 0.5 Bodansky unit per 100 ml. Polyestradiol phosphate was prescribed in a dosage of 40 mg. intramuscularly every two weeks.

In October 1957, the patient entered the Hospital of the University of Pennsylvania for his second and final admission. One week prior to admission he had become confused, lethargic and anorectic. He complained of muscular weakness, especially when climbing stairs. The family had noted a transient "swollen" appearance of his face and abdomen, which had disappeared by the time of admission. Urinary and fecal incontinence had also developed. A review of the past history revealed that for twenty years the patient had had symptoms of chronic constipation which had become worse in the year preceding admission, and for which the patient had treated himself frequently with laxatives. Occasionally treatment would result in diarrhea with 6 to 10 stools daily, but usually

1 to 2 semiformed stools were passed daily. There had been no increase in diarrhea or in the use of laxatives in the weeks prior to admission. There was neither a personal nor a familial history of diabetes mellitus.

On admission, October 24, 1957, the patient was confused, argumentative and uncooperative. Blood pressure was 150/80 mm. Hg, and temperature, pulse and respirations were normal. He appeared well developed and well nourished, but was moderately dehydrated. His skin, facies and fat distribution were normal. Axillary hair was absent and pubic hair was scant. Slight glandular enlargement was palpable in the right breast. The heart was normal except for moderate enlargement. The testes were absent. Rectal examination revealed a stony-hard, irregular, prostatic mass. Patellar and biceps reflexes were absent.

X-ray examinations of the chest showed no evidence of metastatic disease, but there was segmental atelectasis of the left lower lobe. An intravenous urogram demonstrated normal function of the left kidney and moderate hydronephrosis of the right renal pelvis and ureter.

The initial urine specimen showed a neutral reaction, specific gravity of 1.025, albumin 4 plus, sugar 3 plus, and a large amount of acetone; the sediment contained many red blood cells. The concentration of fasting blood sugar was 305 mg. per 100 ml., and of blood urea nitrogen 32 mg. per 100 ml. The serum sodium level was 141 mEq., potassium 1.8 mEq., chloride 85 mEq., and carbon dioxide 50.2 mEq. per liter. Alkaline and acid phosphatase levels were 6.1 and 0.5 Shinowara units per 100 ml. respectively. Serum albumin concentration was 2.7 Gm., and globulin 1.8 Gm. per cent. An electrocardiogram showed depression of the ST segments in the precordial leads and in leads I, II, aVL and aVF. The QT interval was .44 second at a rate of 90 per minute.

Hyperglycemia and glycosuria were controlled with 50 units of regular insulin daily. Intravenous potassium chloride was administered, and later oral potassium supplements were well tolerated. The patient seemed somewhat stronger but continued to be confused and uncooperative despite improvement in the biochemical abnormalities.

The urine (24-hour specimens) was analyzed in order to determine whether potassium depletion was secondary to renal or to gastro-intestinal loss. During the period of intravenous potassium repletion the urinary concentration of potassium was 37–55 mEq. per liter, and of sodium 0.5–2.6 mEq. per liter. Although the serum potassium level was still subnormal, it was thought that these findings were not definitely diagnostic of renal wastage of potassium. Controlled metabolic balance studies were then performed.

Metabolic studies. The patient was transferred to the Metabolic Ward and given a synthetic diet containing less than 5 mEq. of potassium per day and 125 mEq. of sodium. The latter value was chosen to avoid stimulation of aldosterone production, since the urine was to be analyzed for aldosterone content during this period. Feces could not be collected.

Serum values and urinary electrolyte values are listed in Table 1 and Figure 1. The low intake of potassium was continued for fifty-seven hours. The urinary potassium excretion was 70 mEq. the day before potassium deprivation when the intake was 180 mEq. On the second day of deprivation, excretion was 46 mEq. despite a progressive fall in serum potassium concentration. During the remaining nine hours of low intake, the patient excreted an additional 32 mEq. of potassium.

In serial electrocardiograms, ST segment depression and QT prolongation which had been present on admission had reverted towards normal by the time potassium restriction was begun, and did not recur with the redevelopment of hypokaliemia. However, tachycardia developed with a changing cardiac mechanism. Auricular fibrillation, auricular flutter and paroxysmal auricular tachycardia with block all appeared at various

TABLE 1. ANALYSIS OF BLOOD AND URINE DURING POTASSIUM DEPRIVATION AND BEGINNING REPLETION

Period	Potassium intake (mEq.)	Urine*									Blood and serum†					
		Vol. (ml.)	Specific gravity	Solute conc. (mOsm./L.)	pH	Na mEq.	K mEq.	Cl mEq.	NH_3 mEq.	Titr. ac.‡ mEq.	Creatinine (mg./100 ml.)	Na (mEq./L.)	K (mEq./L.)	Cl (mEq./L.)	CO_2 (mEq./L.)	pH§
1 (24 hrs.)	4	1200	1.028	727	5.30	17	54	31	32	26	1.5	142	3.1	97	31.0	7.38
2 (24 hrs.)	4	840	1.025	665	5.30	6	46	10	26	16						
(9 hrs.)	3	575	1.022	659	5.25	17	32	31	15	11	1.8	146	2.9	101	31.3	7.40
3(15 hrs.)	107	770	1.025	681	5.38	7	31	15	23	14	1.8	149	2.6	102	31.0	

* Urine volume and urinary electrolyte values are expressed as total quantity excreted during the period indicated in column 1.
† Blood samples were drawn at the beginning of the period.
‡ Titratable acidity to pH 7.4.
§ "Arterialized" capillary blood.

times and were unaffected by digitalis and quinidine therapy. Because of this complication potassium repletion was begun, but the tachycardia continued.

Fifteen hours after beginning potassium repletion the metabolic studies were discontinued. During the succeeding twenty-four hours fever of 103° F. developed, in associa-

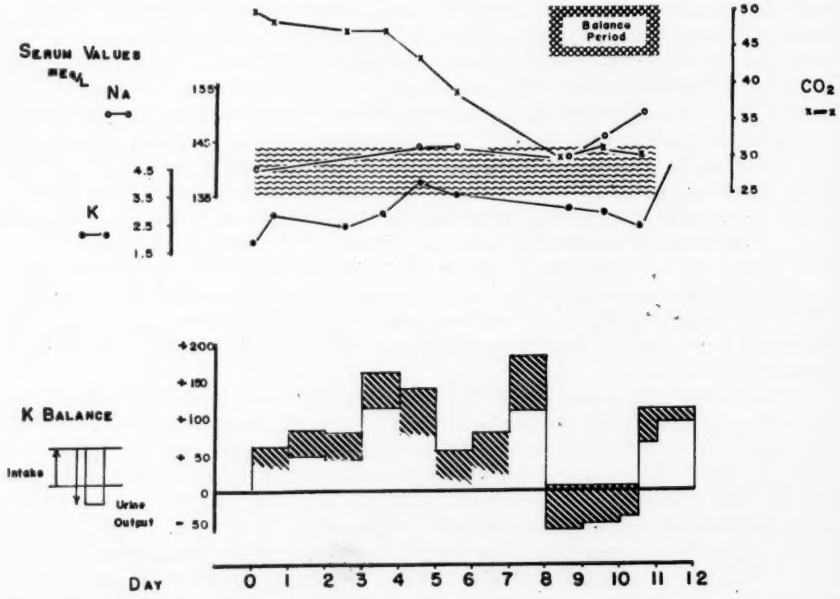

FIG. 1. Serum sodium, potassium and carbon dioxide levels and potassium balance throughout hospital course, in Patient W.K.

tion with signs of pneumonia, cardiac decompensation and shock. The patient died the following day.

Steroid studies. Methods and Results. Soon after admission, urine specimens were analyzed for steroid content. The 24-hour excretion of 17-ketosteroids was 17 mg. and 34 mg., and of 17-hydroxycorticoids 34 mg. and 41 mg. (Porter-Silber method).

Urinary aldosterone and glucocorticoid excretion were then measured during the first two days of the metabolic study. The urine was collected over chloroform and the two 24-hour specimens were pooled. The day following collection, the urine was adjusted to pH 4.5 and incubated with β-glucuronidase (Ketodase) for forty-eight hours at 37° C. After extraction with chloroform and ethylacetate the urine was then adjusted to pH 1, allowed to stand for twenty-four hours and re-extracted with chloroform and ethylacetate. The combined organic phases were washed with saturated bicarbonate and water and evaporated to dryness in a vacuum at a temperature not over 45° C. Preliminary determinations of alpha-ketolic content by the blue-tetrazolium reduction method of Touchstone and Hsu (12) showed an excretion of 41 mg. per twenty-four hours as compared to the normal values of 3–6 mg. The extracts were then subjected to paper chro-

TABLE 2. URINARY ALPHA-KETOLIC STEROIDS (MG./24 HRS.)

Compound	Patient	Normal*
Aldosterone	.06	.005
Cortisol	2.1	.05
Cortisone	1.2	.10
Tetrahydrocortisol and tetrahydrocortisone†	9.2	6.0
Tetrahydrocorticosterone†	.5	.3
Tetrahydro-17-OH-desoxycorticosterone (tetrahydro-S)†	.6	.02

* Maximum values found in normal subjects.

† Semiquantitative estimates.

matography as previously described in detail (13). The cortisone zone which also contained aldosterone and several other alpha-ketolic substances was eluted and rechromatographed in the butyl-acetate: formamide system described by Mattox and Lewbart (14). This afforded the separation of aldosterone from relatively large quantities of unknown alpha-ketols. Aldosterone, cortisol and cortisone were then determined quantitatively by the blue-tetrazolium reduction method (12).

In Table 2 are listed the quantitative measurements of aldosterone, cortisol and cortisone, and semi-quantitative estimates of several of the other alpha-ketols in the urine of this patient. Included also are maximum "normal" values determined by these methods. Aldosterone excretion was 60 μg. per twenty-four hours, which represents more than a tenfold increase over the maximum normal excretion. Excretions of cortisol, cortisone and tetrahydro-S were 2.1, 1.2 and 0.6 mg. per twenty-four hours, respectively: these values represent twelve- to fortyfold increases above normal. A more detailed discussion of the steroid pattern in this case is in preparation (15).

Postmortem findings. Autopsy was performed five hours after death. The remnant of prostatic tissue was completely replaced by a large, firm, yellowish-gray mass invading and compressing the urinary bladder, rectum and right uretero-vesical orifice. The tumor was a poorly differentiated adenocarcinoma with blood vessel invasion. Metas-

tases were found in the lungs, spleen, liver, left kidney, adrenals, pituitary and lumbar vertebrae.

The right adrenal weighed 19 Gm., and the left 20 Gm. The cortex of each adrenal measured from 0.2 to 0.3 cm. in thickness. Microscopically, the adrenal cortices showed a striking hyperplasia of the zona fasciculata, with peculiar large eosinophilic cells which were reminiscent of those in the "androgenic" or X zone of the fetal adrenal and which contained abundant lipoid substance. The glomerular zone was inconspicuous and recognizable only in a few microscopic areas (Fig. 2). There were small foci of metastatic tumor cells in the cortex of each adrenal, similar to those seen elsewhere, but the amount of the tumor tissue was not sufficient to influence the total weight of either adrenal significantly. The pituitary was normal in size, weighing 0.5 Gm. Histologically, acidophilic and chromophobe cells were present in normal proportions in the anterior lobe. Basophilic cells were dimensionally increased and contained vacuoles of variable size and hyaline granules within their cytoplasm (Crooke's change; Fig. 3). One area in the anterior lobe showed a small number of grouped metastatic tumor cells. Sections of the

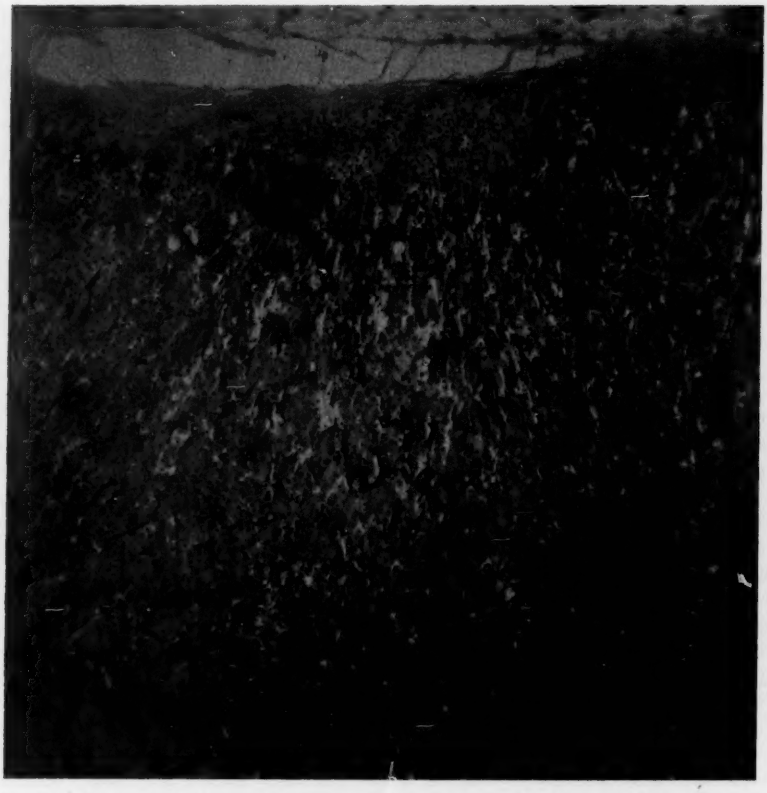

FIG. 2. Photomicrograph of the adrenal showing marked hyperplasia of the zona fasciculata. The arrow points to the inconspicuous zona glomerulosa in the subcapsular area (×50; hematoxylin-eosin stain).

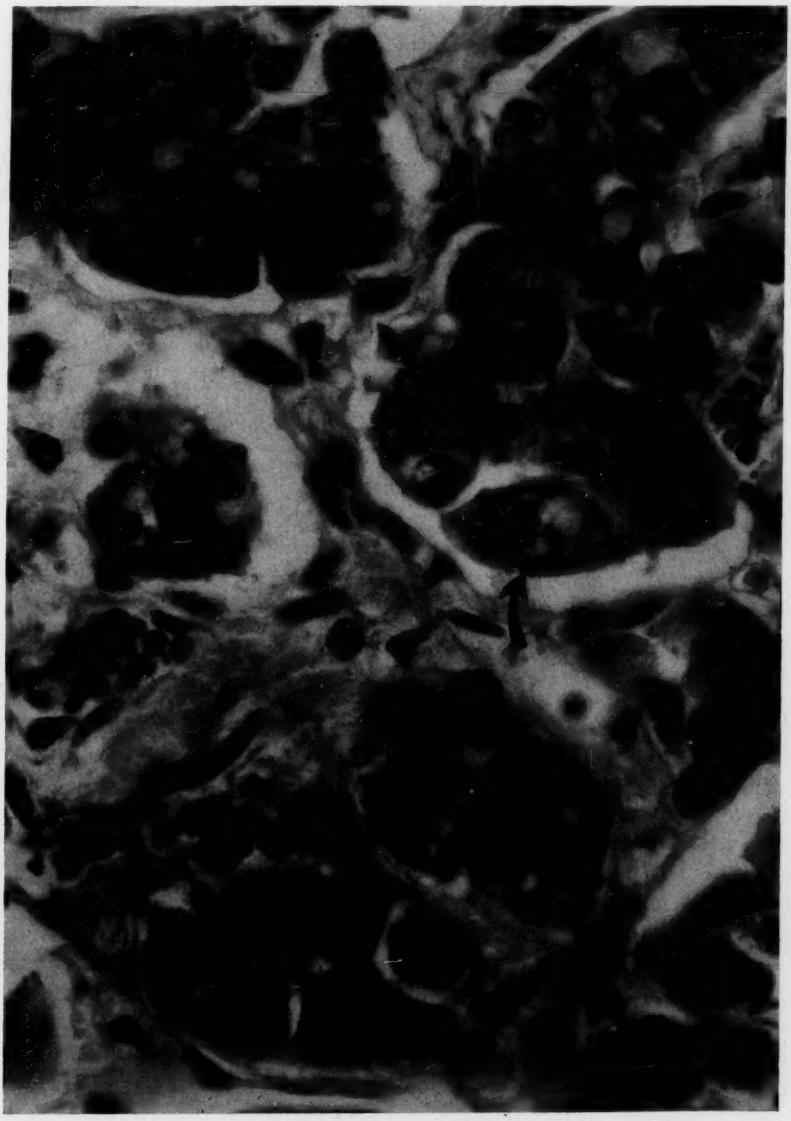

FIG. 3. Photomicrograph of the pituitary gland showing Crooke's change. Note the basophil cells of variable size and shape with characteristic intracytoplasmic vacuoles (×900; hematoxylin-eosin stain).

lumbar vertebral bone marrow demonstrated massive replacement of marrow tissue by similar tumor cells.

Other endocrine organs, including the parathyroid, thyroid and islets of the pancreas, were not remarkable either grossly or microscopically. The kidneys grossly had the coarsely granular surface of nephrosclerosis, and microscopically contained occasional focal areas of cortical stromal fibrosis with sclerosed glomeruli. Although the distal convoluted tubules showed some dilation, the hydropic changes ascribed to potassium depletion were not present. The heart was the seat of small and large foci of myocardial fibrosis, and dissection of the coronary arteries revealed marked atheromatous narrowing of the lumen by thickening of the intima.

A sample of skeletal muscle obtained at autopsy was analyzed for sodium, potassium and chloride content. The results, expressed as mEq. per 100 Gm. of fat-free dry tissue are listed in Table 3, together with normal values (16).

TABLE 3. ELECTROLYTE CONTENT OF SKELETAL MUSCLE OBTAINED POST MORTEM FROM PATIENT W.K. COMPARED WITH THAT OF SKELETAL MUSCLE FROM NORMAL SUBJECTS

	Electrolytes (mEq./100 Gm. of fat-free solids)		
	K	Na	Cl
Patient W.K.	35	18	15.1
Normal subjects*	42.2±2.6	15.1±2.9	8.6±1.8

* Talso *et al.* (16).

DISCUSSION

Urinary steroid excretion

This patient's pattern of elevated glucocorticoid excretion was similar to that in other cases of adrenal hyperplasia (17). The excretion of the reduced form of Substance S, though elevated, was not of the order of magnitude seen in cases of adrenocortical carcinoma (18). The more significant finding is the elevated output of aldosterone; normal excretion of this hormone is the usual finding in patients with adrenal hyperplasia (3, 15, 19) although moderate increases have been reported (19, 20). The reports of normal aldosterone excretion in 2 cases of adrenal hyperplasia associated with lung tumors is perhaps unexpected, since these patients exhibited marked electrolyte abnormalities (3, 11).

It is of interest that in our patient the hyperplasia histologically appeared to be of the zona fasciculata; hyperplasia of the zona glomerulosa was identifiable only in a few microscopic foci. It has been stated that aldosterone production normally takes place in the zona glomerulosa, at least in the rat and the cow (21).

In view of the large quantities of glucocorticoids excreted daily, the absence of clinical features of Cushing's syndrome was striking. The only manifestations detected were hyperglycemia and glycosuria. The most

obvious explanation is that elevated glucocorticoid production had not been present long enough for the development of the expected classic changes. However, Brooks (22) compiled the available data on urinary glucocorticoids in the reported cases of primary aldosteronism and pointed out that although the rates of excretion of these steroids were moderately to markedly elevated in several patients, in none did the usual appearance of Cushing's syndrome develop.

Urinary potassium excretion

Because of the patient's history of constant use of laxatives, it was necessary to establish whether potassium depletion was caused by gastro-intestinal or by renal loss. It is stated that a urinary potassium excretion of less than 20 mEq. per day by a hypokaliemic patient exonerates the kidneys as the pathway for depletion (22), but exceptions to this are to be found in the literature (23, 24). Conversely, the daily excretion of more than 20 mEq. of potassium by a hypokaliemic patient does not necessarily indicate deficient renal conservation, if potassium repletion is under way. Therefore, a trial of potassium deprivation may be necessary to establish renal wasting. The subject of this report showed no tendency to conserve potassium over a 57-hour period of deprivation, indicating that renal loss was more important than gastro-intestinal loss in the development of his potassium depletion.

Acidification of urine

Whereas it is stated that patients with primary aldosteronism characteristically excrete a neutral or alkaline urine (25), this patient excreted an acid urine. During the first two days of potassium deprivation, the urine pH was 5.3 and the average 24-hour excretions of ammonia and titratable acidity were 29 mEq. and 21 mEq., respectively (Table 1). The diet at this time contained a slight excess of fixed cation.

Studies by several investigators (26–29) of mechanisms for the acidification of urine in patients with primary aldosteronism have shown a normal to moderately decreased capacity for the excretion of hydrogen ion; however, a greater-than-normal proportion of this is excreted as ammonia and the urinary pH is increased. This pattern is the same as that seen in experimental potassium depletion (30, 31). Patients with primary aldosteronism recover the capacity to excrete urine with an acid pH when potassium repletion is accomplished either before or after removal of the adrenocortical tumor. It therefore seems probable that this defect results from potassium depletion (27, 29).

A possible explanation for the ability of Patient W.K. to excrete an acid urine may be that potassium repletion was complete before measurements

of urine pH were made. However, hypokaliemia was still present and the potassium content of skeletal muscle obtained post mortem (Table 3) was reduced to a degree comparable to that of the patient reported by Chalmers *et al.* (26).

Concentration of urine

Most patients with potassium depletion are unable to produce a concentrated urine (32). Patient W.K. consistently excreted urine with a specific gravity greater than 1.020. Estimation of the effect of glycosuria on these measurements was not possible during the first days of observation when depletion was most severe, but during the period of potassium deprivation the specific gravity remained high and the urine solute concentration fell only slightly (Table 1). This finding correlates well with the lack of potassium depletion nephropathy noted as autopsy and, as in the case of urinary acidity, might be taken as further evidence that potassium depletion was not profound.

Relationship of adrenal hyperplasia to prostatic carcinoma

The relationship between the adrenal hyperplasia and the carcinoma of the prostate can only be speculated upon, in the light of present knowledge. The other reported cases of clinically apparent adrenocortical hyperplasia co-existing with cancer elsewhere in the body were mainly in patients with pulmonary tumors. Parker and Sommers (9), however, approaching the question primarily from the cancer standpoint, reported a statistically significant increase in the incidence of *histologic* adrenocortical hyperplasia in patients with any type of carcinoma (5.9 per cent) as compared to patients without carcinoma (3.1 per cent). Adrenal hyperplasia was more common when more than one type of carcinoma was present, and when the primary growth was located in the respiratory tract, prostate or gastro-intestinal tract. In a later study limited to cases of prostatic carcinoma, the results were similar (10). None of these patients was known to have had clinical or biochemical evidence of adrenal hyperactivity. The authors stated that there appeared to be no relationship between the incidence of adrenal hyperplasia and the degree of wasting or stress, at least as evaluated by loss of weight. It should be emphasized that the diagnosis of adrenocortical hyperplasia in these studies was based upon the width of the cortex in microscopic sections. It is well known that estimation of the functional status of the adrenal by morphologic techniques is uncertain.

Even if one accepts the foregoing data as evidence that the association of adrenal hyperplasia and carcinoma is more than coincidental, it does not answer the question of whether the adrenal plays a part in the pathogenesis of carcinoma or whether the hyperplasia results from some stimulation,

direct or indirect, of the adrenal by the carcinoma. That the adrenal is stimulated by the carcinoma is suggested by other histologic studies showing an even greater incidence of changes in the anterior pituitary in patients with carcinoma as compared to patients without carcinoma (10, 33, 34). These alterations in the pituitary, which consist of "Crooke's change" and hypertrophy of the "amphophilic" cells, are thought to be definite evidence of adrenal hyperactivity.

In the subject of the present report, the adrenal hyperplasia would seem definitely to have developed sometime following the appearance of the prostatic carcinoma. It is not known whether the metastatic cells in the adrenals and pituitary are an important finding: they were not found in some of the previous cases. It would seem unlikely that metastatic tissue *per se* exerts a stimulatory effect upon the adrenals. If such stimulation existed, adrenal hyperplasia might be expected to be even more common because of the frequency of metastasis to the adrenals, especially from prostatic carcinoma.

Castration and estrogen therapy are still other factors which should be considered as possibly playing a role in the etiology of the adrenal hyperplasia in this case. Under certain conditions castration and estrogenic stimulation lead to adrenal hyperplasia and pituitary adenomata in experimental animals (35). The higher incidence of Cushing's syndrome in females as compared to males (7) suggests that estrogenic activity may play a part in this condition in the human subject.

Acknowledgments

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THE SEMINAL VESICLE-COLCHICINE TEST FOR DETERMINATION OF PITUITARY INTERSTITIAL CELL-STIMULATING HORMONE

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ABSTRACT

A simplified technique for determining the interstitial-cell stimulating hormone (ICSH) content of human urine is described. The method makes use of the increase in the number of mitoses within the seminal vesicles of the immature rat which follows treatment with ICSH or chorionic gonadotropin and colchicine. Preliminary values obtained in normal adults and children of both sexes, and in patients with various pathologic conditions are presented. There is a peak level of ICSH excretion in normal women about the thirteenth day before menstruation. Normal men appear to excrete more ICSH than do normal women (except during mid-cycle). Results were negative in prepuberal children. Of the pathologic endocrine conditions studied, castration was associated with the highest level of ICSH excretion.

SINCE Zondek's (1) pioneer work in 1930, a great deal of effort has been devoted to studies concerning the excretion of gonadotropic hormone in the urine of nonpregnant women. Gonadotropin has been shown to be present under many physiologic and pathologic conditions and much has been learned about the significance of the quantitative variations observed. The data obtained support the view that the pituitary secretes two kinds of gonadotropic hormone—the follicle-stimulating hormone (FSH) which in male test animals acts on the seminiferous tubules, and the interstitial cell-stimulating hormone (ICSH) which acts on the Leydig cells.

The presence of ICSH in the urine has proved difficult to demonstrate, and conflicting results have been obtained (2). However, the development of the kaolin adsorption method has made it possible to prepare urinary extracts with high gonadotropic activity and low toxicity to the experimental animals. According to present knowledge, ICSH stimulates the Leydig cells of the testis to secrete steroids (androgens, and perhaps estrogens) which, in turn, act on the seminal vesicles and the prostate to produce hypertrophy and cellular hyperplasia by mitotic division. ICSH presumably acts directly upon the Leydig cells, because when the hormone is injected into castrated male animals it is not possible to obtain reactions in

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the seminal vesicles and prostate. Therefore, extracts to be assayed for ICSH must not contain androgens or estrogens which act directly on these end-organs.

In 1952 McArthur (3), using the kaolin method of Bradbury *et al.* (4), succeeded in detecting ICSH in the urine of normal women, using hypophysectomized immature male rats as the test animals. The rats received injections of urinary concentrate twice daily during a four-day period, and on the fifth day the weight of the prostate and seminal vesicles was determined. Since 1953 we have been making use of a test developed by us (5) for the determination of ICSH in urine. This method depends upon the increase in mitoses within the seminal vesicles of the immature rat following injection of ICSH and treatment with colchicine. In our opinion, this test possesses some practical advantages over the former method, while retaining its specificity.

It was necessary to find the most convenient schedule for the injection of the urinary extract and the colchicine, and to determine the best time for observing the mitotic reaction. The effects of other variables were also studied. Some of the results obtained by Burkhardt (6) and by us in earlier experiments (7) proved of value in this respect. In making use of normal immature rats we assume that their testes are in a resting condition; that is, that they are not secreting androgens. If, however, the testes or the adrenal cortices are secreting androgenic hormones, the rate must be exceedingly low, since in the majority of control animals it is exceptional to find more than 4 or 5 mitoses per field in tissue sections examined at a magnification of 500×.

METHOD AND MATERIALS

For extraction of urine, the procedure of Bradbury *et al.* (4) was employed with the addition of a final purification step. The final extract as obtained by the Bradbury method is not completely water-soluble, and may produce considerable local reaction with edema. Injection of a 24-hour equivalent of urine occasionally results in death of the animals and in diminished sensitivity. Injection of a 12-hour equivalent frequently does not elicit any reaction, whereas an aliquot of the same extract subjected to additional purification elicits a positive reaction. Moreover, after purification, the extract is completely soluble in the volume of water employed for injection and the test animals appear to react in a manner similar to those treated with preparations of ICSH and human chorionic gonadotropin (HCG). The final purification is accomplished by twice extracting the precipitate obtained by the Bradbury method with 2 cc. of distilled water. The material is centrifuged at 3000 r.p.m. after each extraction and the supernatant solutions are combined. The gonadotropic hormones are precipitated in a centrifuge tube with 10 volumes of acetone, the pH being adjusted to 5.5 if necessary. The tube is left in the refrigerator for twenty-four hours. After centrifugation at 3000 r.p.m. the acetone is discarded. The precipitate, which contains the purified ICSH and FSH, is dried in a vacuum desiccator and is stable for at least a month.

Male litter-mate rats 21 to 23 days of age and weighing 35 to 42 Gm. were employed as the test animals. The extract was dissolved in distilled water in such a volume as to permit the injection of 4-, 6-, 8- or 12-hour equivalents of urine in approximately 0.5 cc. For convenience, we preferred to inject the test material between 8 and 9 a.m. Twenty-seven hours later each rat received an injection of a freshly prepared solution of colchicine (Merck) in distilled water containing 1 to 1.5 μg. of colchicine per Gm. of body weight in a volume of approximately 0.5 cc. When injected in the correct dose, colchicine has the property of stopping mitoses in metaphase. Nine hours after the injection of colchicine the animals were killed. The seminal vesicles were excised and fixed in Bouin's solution overnight. The standard techniques for dehydration and paraffin embedding were followed, but the time ordinarily employed for each step was shortened so as to have the blocks ready approximately four hours later. The sections were cut in a thickness of 5 microns, stained with iron hematoxylin and mounted in balsam.

The colchicine mitoses in at least 10 fields representative of the entire seminal vesicle were counted at a magnification of approximately 500 diameters. The reaction was considered positive when there were at least twice as many colchicine mitoses in the seminal vesicles of the test animals as in those of control rats of the same litter treated only with colchicine. From the moment of injection of the urinary extract until the microscopic examination, the minimal elapsed time was fifty-four hours. In borderline cases an additional assay was performed.

Advantages and disadvantages of the method. The use of hypophysectomized rats is advantageous from a scientific point of view. However, it is impractical in many countries where such animals cannot routinely be obtained. With the seminal vesicle-colchicine method, a high degree of correspondence has been obtained between the results of the test and the clinical diagnosis. This suggests that there is little likelihood of interference from the animal's own pituitary. Our method is practical, inexpensive, easy to carry out, and rapid, requiring only fifty-four hours to obtain a result. It is objective, since various observers obtain similar results, and the slides may be kept for later examination. Its sensitivity is comparable to that demonstrated by Li with purified pituitary ICSH in hypophysectomized rats. A minor disadvantage is the necessity of killing the rats at night. However, we have not been able to devise an injection schedule which is more convenient and at the same time retains maximal sensitivity.

RESULTS

In Table 1 are summarized the results of determinations with purified pituitary ICSH kindly supplied by Prof. C. H. Li, and with the international standard preparation of human chorionic gonadotropin (HCG).

In Table 2 are shown the values obtained for urinary excretion of ICSH in *a*) normal regularly-menstruating women, *b*) normal men, and *c*) normal prepubertal children.

In Table 3 are presented the results obtained in 63 patients with various endocrine disorders related to the adrenals, pituitary and gonads.

TABLE 1. EFFECTS OF PURE ICSH AND THE INTERNATIONAL STANDARD FOR HCG ON THE NUMBER OF MITOSES IN THE SEMINAL VESICLES OF COLCHICINE-TREATED IMMATURE RATS

No. of animals	Amt. injected	Mitoses (test) / Mitoses (control)		
		Avge. per field	Range	Per cent positive
		Pure ICSH		
28	0.1 mg.	10.12	0.82–46.25	89.2
4	0.08	4.03	2.23– 7.45	100.0
3	0.075	10.40	3.52–18.16	100.0
3	0.050	2.30	0.64– 4.59	33.3
9	0	—	—	—
		International standard for HCG		
21	0.5 I.U.	9.0	0.32–45.5	76.2
9	0	—	—	—

DISCUSSION

In previous studies we found that from a threshold level of stimulation up to a maximum level, the number of colchicinic mitoses produced in the seminal vesicle epithelium by androgens is related to the androgenic potency of the preparation employed (14). From Table 1 it may be seen that the number of mitoses is also proportional to the quantity of ICSH or

TABLE 2. URINARY EXCRETION OF ICSH IN PHYSIOLOGIC CONDITIONS

No. of patients	Age	Physiologic status	Reaction for ICSH (with equiv. in hrs. of urine)
8	21 to 23	Normal women	
		ovulatory phase	Pos. (6 to 8 hrs.)
		postmenstrual phase	Neg. (12 hrs.)
		premenstrual phase	Neg. (12 hrs.)
6	17 to 54	Normal men	5 Pos. (10 to 12 hrs.) 1 Pos. (9 hrs.)
8	7 to 9	Normal prepubertal children (3 male, 5 female)	Neg. (24 hrs.)

TABLE 3. URINARY EXCRETION OF ICSH IN VARIOUS ENDOCRINE DISORDERS

No. of patients	Range of age (yrs.)	Diagnosis	Reaction for ICSH (with equiv. in hrs. of urine)*
3	2–51	Cushing's syndrome 1 adrenal hyperplasia (ref. 8) 2 adrenal tumor	 Pos. (4 hrs.) Neg. (12 hrs.)
2	20 & 24	Stein-Leventhal syndrome (ref. 9) After wedge resection in the ovulatory phase	Pos. (12 hrs.) Pos. (9 hrs.)
8	14–32	Gonadal dysgenesis with primary amenorrhea (ref. 10)	2 Pos. (8 hrs.) 3 Pos. (9 hrs.) 2 Pos. (12 hrs.) 1 Pos. (16 hrs.)
2	20 & 21	Secondary amenorrhea	1 Pos. (12 hrs.) 1 Neg. (12 hrs.)
11	14 & 15	Hirsutism (ref. 10) (a) with primary amenorrhea (2 cases)	 1 Pos. (9 hrs.) 1 Pos. (12 hrs.)
	17–28	(b) with secondary amenorrhea (4 cases)—1 adrenal hyperplasia receiving cortisone therapy; 3 under study	1 Pos. (16 hrs.) 1 Pos. (9 hrs.) 1 Pos. (12 hrs.) 1 Neg. (16 hrs.)
	19–36	(c) in normal menstruating women (5 cases) (determinations 9, 11, 12, 13 and 14 days before menstruation)	1 Pos. (8 hrs.) 1 Pos. (7 hrs.) 2 Pos. (9 hrs.) 1 Pos. (14 hrs.)
1	29	Hydatidiform mole (8 days after evacuation)	Pos. (0.012 hr.)
1	19	Pseudohermaphrodite; male chromosomal sex	Pos. (7 hrs.)
16	12–41	Males with hypogonadism (ref. 10) (a) 8 with low ICSH excretion (3 with biopsy showing immature testes, no spermatozoa and very slight development of interstitial tissue)	 5 Neg. (12 hrs.) 1 Neg. (16 hrs.) 2 Pos. (14 hrs.)
		(b) 8 with normal or high ICSH excretion	1 Pos. (8 hrs.) 4 Strongly Pos. (12 hrs.) 3 Pos. (12 hrs.)
4	19–35	Klinefelter's syndrome (all corroborated by biopsy)	2 Pos. (9 hrs.) 1 Pos. (12 hrs.) 1 Pos. (16 hrs.)
1	23	Traumatism of testes; biopsy showed atrophy and fibrosis in one testis	Pos. (12 hrs.)
2	60 & 63	Male climacteric with epistaxis (ref. 11)	1 Pos. (7 hrs.) 1 Pos. (8 hrs.)
1	68	Castrated 7 months before test	Pos. (2 hrs.)
11	3–10	Children with precocious puberty (1 male, 10 female) (refs. 12, 13)	10 Pos. (24 hrs.) 1 Pos. (16 hrs.)

* Some of the positive reactions might have been positive with a smaller number of equivalent hours of urine but were not investigated. In other cases, negative results were also found below the positive amount indicated in the table.

HCG administered. The positive reactions which we have observed (10) following the injection of large quantities of prolactin (2.5 mg.), thyrotropic hormone (2 I.U.) and ACTH (12–20 I.U.) are, in all likelihood, due to contamination with small amounts of ICSH.

The finding of a peak level of ICSH excretion about the thirteenth (from the tenth to the fourteenth) day before menstruation in normal women is in accord with the findings of McArthur *et al.* (15). The lengths of the menstrual cycles in the women studied varied between twenty-four and thirty-three days. Most of the 44 determinations were performed during the ovulatory phase, urinary pregnandiol estimations (16) being employed as a control.

Although the number of men studied was not large, it would appear that their excretion of ICSH was higher than that of women except during mid-cycle. In prepuberal children, the results were negative with 24-hour urine equivalents. Four determinations were made on the urine of a normal 8-year-old girl, but the result was positive in only 1 instance (with a 24-hour equivalent of urine).

Of the pathologic conditions studied, castration was associated with the highest level of ICSH excretion.

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THE OCCURRENCE OF PRE-ECLAMPSIA IN A BILATERALLY ADRENALECTOMIZED WOMAN*

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ABSTRACT

A case is reported of pre-eclampsia developing during the eighth month of pregnancy in a woman who had undergone total bilateral adrenalectomy because of Cushing's syndrome. During the time that she had pre-eclampsia (as manifested by hypertension, edema, albuminuria and an elevated concentration of serum uric acid and confirmed by functional and anatomic changes in the placenta) the levels of plasma and urinary 17-hydroxycorticosteroids were low. At the same time the urinary excretion of aldosterone, also low, was comparable to that reported for normal pregnancies in adrenalectomized women. Hypertension persisted in spite of withdrawal of adrenal steroid therapy and the development of adrenal insufficiency. These events cast doubt on the etiologic role of the adrenal steroids in toxemia of pregnancy.

THE apparent impairment of salt and water metabolism in patients with pre-eclampsia has focussed attention on the adrenal steroids as possible etiologic agents in this disease (1–4). Under certain conditions, pathologic alterations somewhat comparable to those seen in eclampsia, can be produced in experimental animals by the administration of desoxycorticosterone and salt (5). However, results of recent studies (6–8) call for a reconsideration of the role of the adrenal steroids in toxemia of pregnancy. This communication concerns the adrenal and placental function of a bilaterally adrenalectomized woman. She was similar to the patient with Addison's disease reported by Cohen (9), in that pre-eclampsia developed in both. The data provide further evidence that the adrenal steroids do not play a major role in the causation of pre-eclampsia.

METHODS

Plasma free 17-hydroxycorticoids were measured by the method of Nelson and Samuels (10). Plasma conjugated 17-hydroxycorticoids were measured by a modification of the method of Bongiovanni and Eberlein (11). Urinary 17-hydroxycorticoids were

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determined by the method of Glenn and Nelson (12). Pregnanediol excretion was assayed by the method of Guterman and Schroeder (13). Pregnanetriol determinations were made by a modification of the method of Herrmann and Silverman (14) and Bongiovanni and Clayton (15). Chorionic gonadotropin excretion was measured by the ovarian hyperemia reaction of Behnken, Lloyd and Hughes (16). Urinary 17-ketosteroids were determined by a micro modification of the original method of Callow *et al.* (17) using alcoholic KOH and correcting for the nonketonic material with the use of two filters. The values approximate those of the true ketonic material. Aldosterone excretion was determined chemically (18).

CASE REPORT

M. van W., a 38-year-old white female, was admitted to the Syracuse Memorial Hospital on July 3, 1958, because of nausea, hypertension, edema and albuminuria during the third trimester of pregnancy.

TABLE 1. URINARY EXCRETION OF CHORIONIC GONADOTROPIN, PREGNANEDIOL AND PREGNANETRIOL

Date (1958)	Chorionic gonadotropin (rat hyperemia units/24 hrs.) (a)	Pregnanediol (mg./24 hrs.) (b)	Pregnanetriol (mg./24 hrs.) (c)
May 23	10,000	24.8	1.29
June 4	10,000	27.7	1.57
July 2	20,000	18.4	—
July 3	—	11.3	1.72
July 8	20,000	14.0	1.33
July 9	Delivery		

Normal values for this stage of pregnancy:
(a) 1000–4000.
(b) 45–60.
(c) 2–5.

Diabetes mellitus had developed in 1946, making her the fourth member of her family to have the disease. She sought additional medical help in 1952 because of the onset of obesity and amenorrhea. At that time the urinary excretion of formaldehydogenic steroids was within the normal range. Shortly thereafter pulmonary tuberculosis developed and she was hospitalized for fourteen months, during which time a thoracoplasty was performed. In March 1955 she complained of weakness, fatigue and dyspnea in addition to the diabetes, obesity and amenorrhea. Treatment of the diabetes required 65 units of protamine zinc insulin daily. Physical examination revealed a blood pressure of 158/110 mm. Hg, a dorsal fat pad and moon facies. Roentgenograms of the spine were interpreted as demonstrating osteoporosis and a compression fracture of the second lumbar vertebra. On admission to the hospital in August 1955, the blood pressure was 180/120; ophthalmoscopic examination revealed microaneurysms, hemorrhages, exudates and A-V nicking; and the typical habitus of Cushing's syndrome was present. 17-Hydroxycorticosteroid excretion was elevated to 13.9 mg. per twenty-four hours.

A bilateral adrenalectomy was performed; the right gland weighed 12.5 Gm. and the

left 9 Gm. Shortly after discharge, while receiving 30 mg. of hydrocortisone and 0.2 mg. of 9α-fluorohydrocortisone daily, she required additional treatment for adrenal insufficiency. During the subsequent two years the patient was well except for occasional episodes of orthostatic hypotension. The diabetes improved and after March 1957 required no further therapy.

The patient became pregnant in November 1957. At that time her blood pressure was 170/110 and she weighed 137 pounds. By May 6, 1958, the blood pressure had increased to 220/110 mm. Urinary chorionic gonadotropin, pregnanediol and pregnanetriol levels are recorded in Table 1. In the subsequent three weeks, despite the discontinuation of 9α-fluorohydrocortisone the blood pressure remained elevated. During the eighth month of pregnancy she also had pitting edema, headaches, nausea, vomiting, malaise and albuminuria. She was admitted to the hospital.

Physical examination at the time of admission (July 3, 1958) revealed a blood pressure of 210/120 mm., pulse 78 per minute, and weight 147 pounds. No papilledema was present. There was moderate bilateral pedal pitting edema. The lungs were clear, the neck veins flat, and the liver edge not palpable. The uterine fundus was above the umbilicus and the fetal heart beat was present.

Laboratory examinations showed that the urine contained albumin (100 to 300 mg. per 100 ml.), no sugar, 5–10 white blood cells and 3–4 red blood cells per high-power field, and occasional granular casts. The level of serum uric acid was 8.4 mg. per 100 ml., and of blood urea nitrogen (BUN) 23 mg. per 100 ml.; repeated determinations were in the same range. Plasma and urinary steroid values at this and subsequent times are listed in Table 2.

TABLE 2. STEROID LEVELS BEFORE, DURING AND AFTER THE PRE-ECLAMPTIC EPISODE

Date (1958)	Plasma 17-OH-CS (μg./100 ml.)		Urinary 17-OH-CS (mg./24 hrs.)	Urinary 17-KS (mg./24 hrs.)	Urinary aldosterone (μg./24 hrs.)	Hydrocortisone therapy	Comments
	Free	Conj.					
May 22			4.9			10 mg. t.i.d.	
May 28	0.8	1.5					No steroid therapy for 12 hrs.
July 3	3.3	1.8					No steroid therapy for 9 hrs.
July 3 July 4			3.5	2.5	5.1	10 mg. t.i.d.	
July 7 4 P.M.	0	0.96					Last steroid therapy, 6 P.M. July 6
July 7– July 8			1.0	1.2	6.6		13–37 hrs. after last steroid therapy
July 9	*Delivery*						
July 18 8 A.M.	4.6	3.0					Last steroid therapy, 8 P.M. July 17
July 18 2 P.M.	2.2	2.7					25 units ACTH i.v. during 6 hrs.
Sept. 3– Sept. 4				4.5	0.3	10 mg. q.i.d. plus FF	

Initially the patient was treated with bed rest and sedation. The blood pressure remained elevated, with no change in the clinical condition except for the disappearance of edema. Steroid therapy was discontinued and forty-two hours later adrenal insufficiency developed, characterized by a pale, cool, diaphoretic skin and severe nausea. The blood pressure was 210/120 mm. Evidence of adrenal insufficiency disappeared after six hours of treatment with 200 mg. of hydrocortisone intravenously. The next morning, labor was induced while the administration of hydrocortisone was continued, and that afternoon delivery was completed. The baby was alive at birth but died very shortly thereafter. A few hours after delivery the patient was remarkably improved and ate a hearty meal for the first time in weeks. Twenty-four hours after delivery the blood pressure had decreased to 190/110 mm. where it became stabilized while hydrocortisone therapy was being reduced to the maintenance level of 30 mg. per day. On July 18, after two days of preparatory stimulation of the adrenals with 40 units of corticotropin-gel twice daily, an intravenous infusion of 25 units of corticotropin was given over a six-hour period and evoked no adrenal response (Table 2). Hydrocortisone was continued during the preparatory stimulation but withheld after 8 P.M. of the evening prior to the infusion.

During the six months following discharge, while the patient received 40 mg. of hydrocortisone and 0.1 mg. of fluorohydrocortisone daily, the blood pressure ranged between 145/85 and 170/95 mm. Albumin continued in the urine at a 2 plus level. The serum uric acid level gradually decreased to normal and the BUN level remained normal. Weight remained at 137 pounds and there was no edema. Symptomatically the patient was well except for persistent weakness.

Examination of the placenta was performed by Dr. E. C. Hughes. The histologic sections showed large sheets of active cytotrophoblasts with hyperchromatic nuclei. Increased quantities of glycogen were stored in the cytotrophoblasts. There were areas of fibrinoid degeneration in the villi which seemed to cause a disruption in continuity of the villi, many of which were surrounded by a homogeneous fibrinoid material. There also were areas of syncytial degeneration and avascularity of the villi associated with hydropic changes. The overall picture was consistent with toxemia of pregnancy.

Examination of the fetus was performed by Drs. W. D. Alsever and T. C. Wyatt. The baby weighed 1350 Gm. The combined adrenal weight was 7.5 Gm. There were no gross or microscopic lesions other than petechial hemorrhages over the visceral pleura, and diffuse atelectasis.

DISCUSSION

The physiologic role which the increased levels of adrenocortical steroids play in normal human pregnancy is still not defined. The urinary excretion of corticosteroids during normal pregnancy has been extensively studied by various techniques (6, 19–21). The excretion values rise as pregnancy progresses but, for the most part, they remain within the normal range for the nongravid patient. The increased plasma levels of corticosteroids during normal pregnancy may not be due to augmented secretion alone. A delayed rate of inactivation of corticosteroids in pregnancy has been demonstrated (22, 23). A comparable delay in the rate of plasma clearance of infused hydrocortisone has been shown in patients treated with ethinyl estradiol (24). The delay may be due to an altered mechanism for the conjugation of tetrahydrocortisone and presumably tetrahydrocortisol

with glucuronic acid (25). High plasma levels of corticosteroids are not essential for a normal pregnancy. Women with adequately treated adrenal insufficiency (either spontaneous or as the result of bilateral adrenalectomy) can have normal pregnancies (26). In normal pregnancy the urinary excretion of aldosterone rises steadily to levels considerably above the range observed in the nonpregnant patient (7).

In pre-eclampsia the urinary excretion of corticosteroids is said to be increased more than in normal pregnancy (19, 20, 27). These observations have not been confirmed by Martin and Mills, who used the Norymberski technique (6). It has been reported that in toxemia the urinary excretion of salt-retaining substances is increased (28–31), but more refined techniques have demonstrated that the excretion of aldosterone is not augmented and may be even less than in the normal pregnant woman (7, 32–35). As a result of isotope dilution studies it has been concluded that excessive sodium retention is not the cause of the edema of pre-eclampsia (8).

In patients without endogenous adrenal steroids who are maintained with a fixed regimen of replacement therapy during pregnancy there is a slight increase in urinary excretion of (36–37), and a progressive rise in the plasma levels (38) of Porter-Silber chromogens. When the pregnant woman with no adrenal function is not receiving cortisone or hydrocortisone replacement therapy, no corticosteroids are detectable in either the urine (22, 36) or the plasma (37, 39). No aldosterone was found by Baulieu *et al.* (39) in the urine of a pregnant patient with Addison's disease, whereas Laidlaw *et al.* (40) reported a urinary excretion of between 1.0 and 4.4 μg. of aldosterone per twenty-four hours in 2 pregnant adrenalectomized women.

The clinical picture presented by our patient was in all respects that of of typical pre-eclampsia superimposed on an underlying cardiovascular disease. The placental changes, both physiologic and anatomic, were consistent with this diagnosis. The pre-eclampsia developed while the patient's intake of hydrocortisone, 9α-fluorohydrocortisone and salt remained constant. The elevated blood pressure persisted after the discontinuation of the fluorohydrocortisone. At that time there was the usual pre-eclamptic pattern of elevated excretion of chorionic gonadotropin and decreased excretion of pregnanediol. As the disease progressed, the blood pressure rose even higher. Peripheral edema, albuminuria and hyperuricemia developed. At that point the excretion of aldosterone in the urine was in the same range as that found by Laidlaw *et al.* (40) in well maintained bilaterally adrenalectomized patients who had normal pregnancies. Although the edema disappeared with bed rest, the vomiting, hypertension, albuminuria and hyperuricemia persisted. Twenty-two hours after cessation of all adrenal steroid therapy the concentration of plasma 17-hydroxycorticosteroids was

negligible. Forty-two hours after cessation of all therapy (while the patient was still hypertensive) adrenal insufficiency developed, requiring the administration of large amounts of hydrocortisone for its correction. Subsequent to the delivery of the fetus and placenta, during which time large amounts of hydrocortisone were administered and circulating steroid levels must have been high, rapid clinical improvement occurred. The possibility that accessory adrenal tissue might have become active during pregnancy seems unlikely, since there was no increase in plasma 17-hydroxycorticoid levels during administration of corticotropin in the subsequent nonpregnant state.

The fact that during pregnancy this woman excreted amounts of aldosterone which, although low, were ten times greater than the excretion during her nonpregnant state suggests the possibility of placental or fetal formation of aldosterone, or an alteration in steroid metabolism. It is of interest that our patient and an adrenalectomized patient reported by Laidlaw *et al.* (40) who excreted modest amounts of aldosterone during pregnancy and negligible amounts subsequently, were receiving hydrocortisone, whereas the patient of Baulieu *et al.* (39) who did not excrete any detectable amounts of aldosterone during or after pregnancy was not taking hydrocortisone. This would lend weight to the suggestion of Laidlow *et al.* (40) that, during pregnancy, hydrocortisone may be metabolized in a different manner than when the patient is not pregnant.

Pregnanetriol excretion values approximated the upper range of normal for nonpregnant females (15) and were below the range reported in normal pregnant women near term (41). In women with Addison's disease undergoing uneventful pregnancy, pregnanetriol excretion values are in the range for normal pregnant women (37, 41). One can speculate that the toxemic process might have interfered with pregnanetriol metabolism.

Our case and that of Cohen (9) appear to demonstrate that pre-eclampsia can develop and persist without the presence of maternal adrenal tissue and with depressed levels of adrenal steroids. This finding suggests the possibility that the causative agent may be some, as yet unknown, substance formed by the placenta and that the role of adrenal steroids in the toxemia of pregnancy is, at most, "permissive" in nature.

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GONADAL DYSGENESIS IN THREE SISTERS

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ABSTRACT

Elevated levels of serum acid phosphatase are recorded in 3 sisters with gonadal dysgenesis. The clinical features included primary amenorrhea occurring in feminine-appearing women who did not show other stigmata of the Bonnevie-Ullrich-Turner syndrome. The nuclear chromatin sex pattern in the cells of the skin, blood, and vaginal smear was female. The presence of "marked osteoporosis and vertebral epiphysitis" was noted in all. It is suggested that these bony changes constitute an incomplete form of osteogenesis imperfecta, which probably accounts for the abnormally high levels of serum acid phosphatase.

SINCE the earlier descriptions (1) of the Bonnevie-Ullrich-Turner syndrome, namely, gonadal dysgenesis with short stature and associated congenital abnormalities, an increasing number of variations of syndromes based upon gonadal dysgenesis have been described.

Swyer (2) described patients with normal height, eunuchoidal proportions, feminine appearance, and female genitalia. Secondary sexual characteristics were not developed, nor did these patients menstruate. The nuclear chromatin pattern was male. None of the congenital abnormalities described in the Bonnevie-Ullrich-Turner syndrome was present.

Hoffenberg and Jackson (3) described a case with clinical features similar to those in Swyer's cases, but with a female nuclear pattern. Laparotomy revealed primitive gonadal ridges, which histologically showed ovarian stroma without follicles but containing aggregates of cells resembling ovarian hilar cells.

The purpose of this communication is to describe the foregoing syndrome of "Gonadal dysgenesis in normal looking females with female nuclear pattern" as it occurred in 3 sisters. The genetic aspects and the association of certain skeletal and biochemical changes will also be described.

CASE REPORTS

Case 1

A 23-year-old housewife, married for two years, was admitted to the Johannesburg General Hospital with a history of having slipped and fallen heavily on her left hip.

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X-ray examination revealed a slipped epiphysis of the neck of the left femur. The femur was osteoporotic. She had never menstruated, nor received hormonal therapy. Childhood had been normal. A gynecologist had told her at the age of 16 that she would never bear children. She had a normal heterosexual outlook and normal libido. There was no history of dysparunia. She was one of 11 siblings. Seven older siblings (3 brothers and 4 sisters) were married and all had children; a younger sister was pregnant. None had any congenital abnormalities. However, 2 other sisters, aged 25 and 26, had never menstruated. These 2 sisters and the present subject were consecutive births. The rest of the family pedigree was not remarkable.

The general appearance of the patient was feminine, but her proportions were eunuchoidal (Fig. 1). Her height was 65 inches (165 cm.), span 70 inches (177 cm.), measurement from pubis to crown 30 inches (76 cm.), measurement from pubis to heel 35 inches (89 cm.), and weight 120 pounds. Her hands were strikingly long and slender. The voice was of normal female pitch. Breasts were small, and without areolar pigmentation. Axillary and pubic hair was scanty, the latter being feminine in distribution. Otherwise, the results of general examination were noncontributory.

On pelvic examination, the clitoris was found to be slightly enlarged. The labia majora and minora were small. The vagina was of normal size and shape, the cervix was conical, and the uterus was small. No adnexae were felt. The cellular picture in a vaginal smear indicated a moderately atrophic state of the vaginal epithelium.

Laboratory investigations: The hemogram and serum electrolyte levels were normal, The Ide test for syphilis yielded negative results. The level of serum cholesterol was 155 mg. per 100 ml., protein-bound iodine 9.8 μg. per 100 ml., calcium 5.4 mEq. per liter, phosphorus 4 mg. per 100 ml., alkaline phosphatase 8.4 King-Armstrong (K.A.) units per 100 ml. and acid phosphatase 5.7 K.A. units per 100 ml. (normal range, 1.0–3.0 K.A. units). Urinary 24-hour hormonal excretions were as follows: follicle-stimulating hormone 48 mouse units (usual range, 6–12 mouse units), 17 ketosteroids (measured as dehydroisoandrosterone) 15.6 mg. (normal range, 8–15 mg.), 17-hydroxycorticosteroids (measured as free cortisone) 7.3 mg. (normal range, 1.1–8.6 mg.).

In the cells of a skin biopsy specimen, blood smear and vaginal smear (Dr. S. Klempman) the nuclear chromatin pattern indicated a genetic female. Radiologically, bone age was estimated to be 14 years. There was generalized osteoporosis with epiphysitis of the annular epiphyses of the mid-dorsal vertebrae.

Because of the bony changes and the possibility of delay in union of the slipped femoral epiphysis, the patient was given 1 mg. of stilbestrol daily. Two months later the breasts had increased in size and the areolae were pigmented. A plaster spica was applied for treatment of the left femur and was retained for three months; the fracture healed well.

Case 2

A married 27-year-old housewife was admitted for investigation of primary amenorrhea. The childhood history was normal. At the age of 19 years she consulted a gynecologist and was given hormonal therapy. This was followed by breast development and appearance of axillary and pubic hair. For eighteen months prior to being seen by the authors, the patient received endocrine therapy and experienced uterine bleeding each month. She had a normal feminine heterosexual outlook and normal libido. There was no history of dysparunia.

The general appearance was that of a rather thick-set female of eunchoidal proportions (Fig. 2). Her height was 66¼ inches (168 cm.), span 72¼ inches (184 cm.), measure-

ment from pubis to crown 32 inches (81 cm.), measurement from pubis to heel 34¼ inches (87 cm.), and weight 180 pounds. Her hands were strikingly long and slender. The voice was of normal female pitch. The breasts were pendulous, the right more than the left, but no true breast tissue was palpable. The areolae were pigmented as a result of previous endocrine therapy. Axillary hair was abundant; pubic hair was scanty but of normal female distribution. The rest of the findings on general examination were noncontributory.

Pelvic examination showed the clitoris, labia majora and labia minora to be small. The vagina was of normal size and shape, the cervix was small and conical, and the uterus

FIG. 1. *Case 1.*, after stilbestrol therapy.

FIG. 2. *Case* 2.

was small. No adnexae were felt. The vaginal smear showed some evidence of estrogenic activity.

Laboratory investigations: The hemogram and serum electrolyte levels were normal. The Ide reaction for syphilis was negative. The level of serum cholesterol was 184 mg. per 100 ml., protein-bound iodine 6.1 μg. per 100 ml., calcium 4.9 mEq. per liter, phosphorus 4.7 mg. per 100 ml., alkaline phosphatase 7.6 K.A. units per 100 ml., and acid phosphatase 9.1 K.A. units (repeated, 4.7 units). Urinary 24-hour excretion of follicle-stimulating hormone was 24–48 mouse units. 17-Ketosteroid and estrogen excretions and their response to chorionic gonadotropin are shown in Table 1. The nuclear chromatin pattern in cells of the skin and blood (Dr. S. Klempman) indicated a genetic female. Radiologically, bone age was estimated to be 23 years. The dorsal vertebrae were osteoporotic and showed early epiphysitis of the annular epiphyses.

TABLE 1. EFFECT OF CHORIONIC GONADOTROPIN ON 24-HOUR URINARY OUTPUT OF 17-KETOSTEROIDS, ESTRONE AND ESTRIOL

There was no change in 17-KS, but a rise in estrone and estriol excretion. No estradiol was found in any specimen.

Date (1957)	Case 2			Case 3		
	17-KS (mg.)	Estrone (μg.)	Estriol (μg.)	17-KS (mg.)	Estrone (μg.)	Estriol (μg.)
Aug. 1	7.4	0	4.9	18.6	3.2	4.9
Aug. 2	8.2	0.7	3.9	11.8	2.6	3.4
(8 A.M.) *Chorionic gonadotropin, 1500 units i.m.*						
Aug. 3	6.8	11.1	6.1	9.0	12.7	17.8
(8 A.M.) *Chorionic gonadotropin, 1500 units i.m.*						
Aug. 4	7.4	5.3	7.6	8.9	11.0	4.5
Aug. 5	7.1	5.9	10.6	7.1	13.8	4.3
Aug. 8	10.0	2.0	9.2	11.4	4.1	5.2

Estrogen fractions were determined according to the method of Brown (*Biochem. J.* **60**: 185, 1955), and the 17-KS determinations were made according to a slight modification of the method recommended by the Medical Research Council Committee on Clinical Endocrinology (*Lancet* **2**: 585, 1951).

Case 3

A 25-year-old married housewife also presented with primary amenorrhea. She gave a history of measles and mumps during childhood. At the age of 19 years she consulted a gynecologist because of lack of breast development and amenorrhea. Pubic and axillary hair were scanty at that time. She was given intermittent courses of estrogen and progesterone, resulting in a few "scanty periods" and moderate breast development with areolar pigmentation. Following her marriage at the age of 20 years, she had two bouts of pyelocystitis. She complained of dysparunia, which appeared to be relieved while receiving hormonal therapy. She had a normal feminine heterosexual outlook and normal libido.

The general appearance was feminine, with eunuchoidal proportions (Fig. 3). Her height was $66\frac{3}{4}$ inches (169 cm.), span 73 inches (185 cm.), measurement from pubis to crown $31\frac{1}{4}$ inches (79 cm.), measurement from pubis to heel $35\frac{1}{2}$ inches (90 cm.), and weight 135 pounds. Her hands were strikingly long and slender. The voice was of normal female pitch. Breasts were poorly developed; areolar pigmentation was present as a result of previous endocrine therapy. Axillary and pubic hair was scanty, the latter having a female distribution. The rest of the findings on general examination were noncontributory.

Pelvic examination showed the clitoris to be small, and the labia majora and minora poorly developed. The vagina was conical, and the cervix and uterus were small. No adnexae were felt. Vaginal smears were markedly hypoestrogenic.

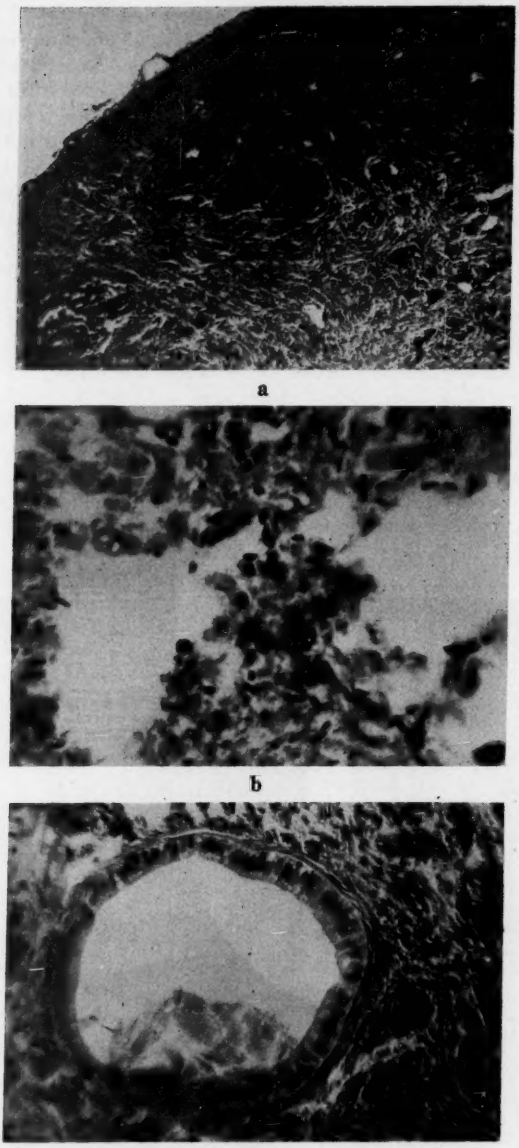
Laboratory investigations: the hemogram and serum electrolyte levels were normal. The Ide reaction for syphilis was negative. The level of serum cholesterol was 225 mg. per 100 ml., protein-bound iodine 7.6 μg. per 100 ml., calcium 5.3 mEq. per liter, phosphorus 4.8 mg. per 100 ml., alkaline phosphatase 39 K.A. units per 100 ml. (repeated, 33 and 27 units), and acid phosphatase 10.5 K.A. units per 100 ml. (repeated, 8.4 units). The 24-hour urinary excretion of follicle-stimulating hormone was 12–24 mouse units. Urinary 17-ketosteroid and estrogen excretions and their response to chorionic gonadotropin are listed in Table 1. Examination of cells in a skin biopsy specimen and blood smears (Dr. S. Klempman) showed a female chromatin pattern. Radiologically, bone age was estimated to be 23 years. The dorsal vertebrae were osteoporotic and epiphysitis of the annular epiphyses was evident. There was wedging of D5 (Fig. 4), apparently asymptomatic.

Laparotomy (Dr. L. G. R. van Dongen) revealed an infantile sized uterus 2.5 cm. in length, and Fallopian tubes 6 cm. in length. Typical primitive gonadal ridges were present from which sections were taken. Dr. S. Shippel reported:

"Sections show tissue consisting of cortex and medulla. The cortex consists of connective tissue and muscle on the surface, and a stroma which bears some resemblance to ovarian stroma (Fig. 5a). Occasional small islands of somewhat luteinized cells occur in the stroma, which resemble theca cells (Fig. 5b). Only one structure which is rather degenerate bears a superficial resemblance to a primordial follicle. In the medulla there are a number of tubules which from their appearance are most likely of mesonephric origin" (Fig. 5c).

FIG. 3. *Case 3.*

FIG. 4. *Case 3.* Osteoporosis of dorsal vertebrae, with epiphysitis of annular epiphyses and wedging of D5.



a

b

c

FIG. 5. a) Low-power photomicrograph of a ? gonad, showing surface epithelium of serosal origin, a very narrow cortex, and a rather large medulla (×35). b) High-power view of islands of luteinized cells in the stroma, resembling theca or interstitial cells (×255). c) High-power view of a tubule in the medulla surrounded by smooth muscle. This tubule is probably of mesonephric origin (×125).

DICSUSSION

The experimental work of Jost (4) in rabbits and Raynaud and Frilley (5) in mice established that the female form is basic or neuter, and male differentiation occurs only in the presence of functioning testes during embryonic development. Grumbach *et al.* (6), applying these data, suggested that the syndrome of gonadal dysgenesis might be produced by a primary intra-uterine disturbance affecting the germ plasm between the eighth and tenth week, and postulated a deleterious environmental agent of unknown nature. The hypothesis is possibly supported by the superficial resemblance of some of the malformations seen in this syndrome to those seen as a result of fetal rubella, *e.g.*, cataract, cardiac defects and deafness.

The fact that gonadal dysgenesis has been described in twins (7), in a patient whose two paternal aunts were similarly affected (8), and now in 3 sisters, may perhaps suggest that the syndrome is genetically determined. Hoffenberg and Jackson (3) explain the different clinical syndromes observed on the basis of 3 closely related genes on 1 chromosome—"I" (intrauterine hypogonadism), "S" (shortness of stature) and "A" (associated congenital abnormalities, *e.g.*, musculoskeletal, cardiac, renal and ocular). There is, however, very little evidence to support this ingenious hypothesis. Furthermore, population studies are not grounds for postulating linkage; this requires family trees, and these are not available.

Our 3 cases all had radiologic evidence of "osteoporosis, retarded bone age and vertebral epiphysitis." The nature of the bone lesions frequently observed in gonadal dysgenesis has been disputed. Albright and Reifenstein (9) attribute the bone lesions to absence of sex hormones, which are intimately concerned in the laying down of bone matrix and the closure of the epiphyses. Lack of these hormones during childhood and adolescence is manifested by postponement of skeletal maturity. Thus there is continued proliferation of epiphyseal cartilages, particularly in long extremities in comparison with the trunk, resulting in eunuchoidal proportions. Many patients with the Bonnevie-Ullrich-Turner syndrome have osteoporosis, retarded bone age and eunuchoid proportions, but are not tall, possibly because of a genetic shortness of stature. In the absence of the latter, the patient will be tall or of normal height, as in our cases.

Recently various authors (10, 11) have suggested that the so-called "osteoporosis" described in association with gonadal dysgenesis is an incomplete form of osteogenesis imperfecta and constitutes part of the inherited syndrome. Hoffenberg and Jackson (12) support this view, and noted the presence of blue sclera (a well described feature in osteogenesis imperfecta) in certain of their cases of gonadal dysgenesis.

From the biochemical standpoint, the particular interest of the authors' cases of gonadal dysgenesis lies in 1) the presence of an elevated level of

serum acid phosphatase, and 2) data on urinary estrogen excretion obtained in detailed studies performed in 2 cases.

This is apparently the first report of a raised level of serum acid phosphatase in gonadal dysgenesis. A high level of serum acid phosphatase is classically associated with metastasizing carcinoma of the prostate, but it has also been described in conditions associated with bone resorption (13). Gebala (14) described a series of cases of osteogenesis imperfecta in which elevated serum acid phosphatase levels were consistently present. If we accept the modern view that the "osteoporosis and epiphysitis" present in these 3 sisters indicate an incomplete form of osteogenesis imperfecta, it is not unreasonable to relate the elevated serum acid phosphatase levels to the presence of bone lesions.

Another unusual biochemical feature was the presence of a significantly raised concentration of serum alkaline phosphatase (39 K.A. units) in Case 3. Other than the bone lesions noted ("osteoporosis"), no cause could be ascertained for this abnormality. A high serum alkaline phosphatase level, however, is a most unusual finding in "osteoporosis" in the absence of fracture.

Both estrone and estriol were found in the urine of the 2 subjects investigated (Cases 2 and 3). Estradiol was not detected in any of the specimens examined. Following stimulation with chorionic gonadotropin (1500 units by intramuscular injection on two successive days) there was a rise in the excretion of estrogen, which returned rather slowly to pretreatment levels. As there was no ovarian tissue in Case 3, the rise of estrogen excretion after administration of chorionic gonadotropin suggests that there was a source of estrogen other than ovarian tissue which was under the influence of chorionic gonadotropin. It is not unreasonable to postulate that the adrenal cortex was the source.

Excretion of 17-ketosteroids by all 3 subjects was normal. There was no evidence of any increase or alteration of 17-ketosteroid excretion following administration of chorionic gonadotropin.

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Letters to the Editor

MASCULINIZATION OF THE FEMALE INFANT ASSOCIATED WITH ESTROGENIC THERAPY ALONE DURING GESTATION: FOUR CASES*

To the Editor:

Masculinization of the female infant has in some instances been attributed to the antenatal exposure of the fetus to androgens from several sources. Virilizing hormones may arise: 1) from the fetal adrenal in the adrenogenital syndrome due to congenital adrenocortical hyperplasia; 2) from virilizing tumors in the mother (arrhenoblastoma); 3) from the administration of primarily androgenic hormones to the mother during pregnancy; and 4) from certain "progestins" administered to the mother during pregnancy. This report concerns 4 female infants and children with some degree of masculinization who were recently seen in 3 different clinics. In each instance it was determined that the mother had received diethylstilbestrol *alone* during pregnancy. There were no other factors discovered to account for these changes and it was considered that the estrogenic hormone probably played some role in producing the effects described.

CASE REPORTS

Case 1

J.L. was seen at The Children's Hospital of Philadelphia, when $4\frac{2}{3}$ years of age, because of abnormal genitalia present since birth. The mother had had 4 previous pregnancies, 3 of which had terminated spontaneously between three and six months' gestation. The fourth was uneventful and productive of a "perfectly normal" female, now aged $7\frac{1}{2}$ years. The patient under consideration was the product of a fifth pregnancy, during which diethylstilbestrol had been administered to the mother from the seventh to the twentieth week, in a dosage of 5.0 to 50.0 mg. daily. Progesterone or "progestins" had not been used. No other unusual hormones or medications had been administered to the mother. At the time of the first examination, the patient was in the fiftieth percentile for both height and weight. She displayed no evidences of masculinization aside from the changes in the genitalia. The clitoris was obviously enlarged, measuring 2.5×1.0 cm., with a redundant foreskin and a dimple on the glans which failed to admit a fine

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catheter. The labioscrotal folds were fused to the base of the phallus (Fig. 1), despite an operation which the mother stated had been performed at an earlier age in order to open the vagina. The patient's mother had at no time experienced virilization, but she was not further studied because of severe neurosis and undue concern for her child. A vaginogram performed on the patient via the small orifice, revealed a vagina, cervix uteri, and urethra; the latter was slightly displaced posteriorly. The nuclear chromatin pattern was female. No further studies were permitted.

FIG. 1. *Case 1.*

FIG. 2. *Case 2.*

Case 2

B.R. a 7-year-old girl, was seen at The Children's Hospital of Philadelphia because of an enlarged clitoris present since birth. The parents had always been aware of this and had repeatedly procrastinated because of what they believed to be unbearable prospects. The mother's first 2 pregnancies terminated at the third and fourth months, respectively. Her third pregnancy was successful, presumably without treatment, and productive of a normal male, now aged 13 years. The patient was born after a pregnancy characterized by bleeding during the fourteenth week, for which reason the mother received diethylstilbestrol, 25 mg. daily, through the twentieth week. No other hormones, including progesterone or its analogues, had been employed. On physical examination, the child was normal in size for her age (about the sixtieth percentile for height and weight) and as yet sexually immature. There was no hirsutism. The significant physical findings were confined to the genitalia (Fig. 2). The clitoris was obviously much enlarged, measuring 4.5×1.9 cm., with a firm corpus and reduntant foreskin. There was only minimal fusion posteriorly to the labioscrotal folds, the vaginal orifice appeared

adequate, and the urethral orifice was easily seen in its normal position. Nonetheless a vaginogram was performed, which revealed a normal sized vagina and cervix uteri. The uterus, normal in size, was palpable on rectal examination. The bone age was normal. The nuclear chromatin pattern was female. Excretion of 17-ketosteroids, determined on several specimens of urine, ranged from 0.6 to 3.5 mg. per twenty-four hours. The mother was in good health and did not exhibit virilization during pregnancy. Her urine contained 10.7 mg. of 17-ketosteroids and 0.8 mg. of pregnanetriol in a 24-hour specimen at the time the patient was first observed by us.

FIG. 3. *Case 3.*

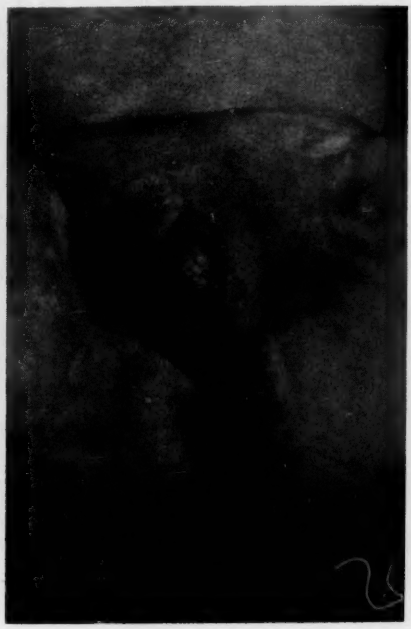

FIG. 4. *Case 4.*

Case 3

C.J. was seen at the Babies Hospital in New York City when 2 months of age, because of abnormal external genitalia. Owing to a miscarriage at the third month during the only previous pregnancy, the mother received diethylstilbestrol, 10 mg. daily, during the thirteenth week of this pregnancy; the dosage was gradually increased to 75.0 mg. daily during the twenty-fifth week. No other hormones were administered. The patient was born at the end of the thirtieth week of gestation. The birth weight was 1150 Gm. When examined at 2 months of age she weighed 2380 Gm. At this time the pertinent physical findings were limited to the external genitalia. The clitoris was enlarged and measured 3.2×1.0 cm.; it was covered by a prominent preputial fold (Fig. 3). The labia majora were fleshy and conspicuous but there was no fusion. Separate urethral and vaginal orifices were identified. Oral mucosal smears showed female-type nuclei and the 24-hour excretion of 17-ketosteroids was 0.1 mg. A 24-hour specimen of the mother's urine was examined and contained 4.2 mg. of 17-ketosteroids and 6.9 mg. of corticoids.

Case 4

E.Y., at 7 days of age, was admitted to St. Christopher's Hospital for Children in Philadelphia because of abnormal genitalia. The mother was treated with 5 mg. of diethylstilbestrol twice daily between the fourth and ninth week of pregnancy because of severe abdominal cramps. No other hormones were administered during pregnancy and the mother had not had any signs of virilization. The cramps subsided a few days after institution of therapy. The patient had a brother and sister who were normal and there was no family history of congenital abnormalities. Physical examination revealed clitoral enlargement (1.5 cm.) and labioscrotal fusion to the base of the phallus (Fig. 4). The 24-hour 17-ketosteroid excretion was 0.6 mg. The chromosomal sex was female. Injection of radio-opaque media into the urogenital sinus resulted in filling of the bladder, but a vagina was not demonstrated. Exploratory operation revealed a normal uterus, tubes and ovaries—the latter proved by biopsy. There has been no progressive virilization of the infant and growth and development have been normal.

DISCUSSION

Congenital adrenocortical hyperplasia, arrhenoblastoma in the pregnant woman, and the administration of primarily androgenic hormones as factors in the masculinization of the female infant have been considered in a monograph by Grumbach and Barr (1). More recently, Wilkins *et al.* (2), Jones (3), and Hayles and Nolan (4) have called attention to similar effects produced by the administration of certain progestins. Wilkins *et al.* (2) pointed out the similarity in structure of some progestins and androgenic hormones; however they attributed their findings to some unusual metabolic disposition of these compounds which may produce androgenic substances. It should be noted that 10 of 21 cases reported by Wilkins and associates (2) received estrogens (diethylstilbestrol in 9) as well as progestins.

The incrimination of predominantly estrogenic hormones in the 4 cases reported here seems paradoxical. However there is some basis in experimental embryology for this occurrence. Although it is difficult to establish these changes as due to diethylstilbestrol, all other known factors have been ruled out. The mothers received no other hormones. The true adrenogenital syndrome was adequately ruled out in each of the children on both laboratory and clinical grounds; in none of the patients was masculinization progressive. None of the mothers presented clinical evidence of virilization and hence it appears unlikely that endogenous maternal hormones, in abnormally large quantity, played any role. In 2 of the mothers who were studied, the daily excretion of 17-ketosteroids was normal.

Much attention has been given in experimental embryology to the alteration of sexual development by the administration of estrogenic or androgenic hormones during gestation. Ordinarily, attention has been focused on the ability of one group of hormones to bring about homologous effects on fetuses of the opposite sex. In 1939 Greene *et al.* (5) reported

paradoxical effects of estrogens on the sexual development of the female rat. As expected, when estradiol was given to pregnant rats, the male offspring were feminized, with inhibition of development of the epididymis, vas deferens, seminal vesicle and prostate; the vagina, parts of the uterus, and nipples persisted. Surprisingly, the female offspring showed paradoxical effects; there was inhibition of development of the lower vagina, with a small opening at the caudal base of the cleft phallus; in addition, there was inhibition of the ovarian capsule, partial preservation of wolffian-duct structures, and even seminal vesicle formation in a few. Later, Greene and his colleagues (6) employed diethylstilbestrol as well as estradiol, presumably with similar results. Moore (7) reported the stimulation of wolffian ducts by estrogens in the oppossum, and this occurred in both sexes—an observation differing somewhat from that in the rat. However, enlargement of the clitoris was not described in these experiments. Any explanation for these curious results was lacking. A direct teratogenic effect of the large doses of estrogens on the genital primordia must also be entertained.

The work of Lacassagne in 1927 (8), demonstrating an increase in adrenal size following the administration of estrogens to rats, has been repeated by many others with similar results. This observation has also been recorded for the guinea pig, mouse, cockerel and other species. In 1945, Vogt (9) demonstrated that the administration of hexestrol (a synthetic estrogen, related to diethylstilbestrol) caused a complete loss of lipids in the adrenal gland of the rat within five days, with an increase in the size of the organ and general intolerance to stress akin to adrenocortical insufficiency. Subsequently Vogt (10) described a diminution in the secretory rate of corticosterone by the rat's adrenal following the administration of hexestrol; progesterone alone did not produce this result. The rabbit was not so affected by hexestrol. Clayton and Hammant (11) recently described changes in the adrenal of the rat (but not the guinea pig) following administration of stilbestrol, with an increase in the size of the gland. They suggest interference with the normal secretion of steroids. Witschi (12) described adrenocortical hyperplasia in the larvae of 3 species of Rana when estrone, estradiol, ethinyl-estradiol or equilinin were added to the water in the aquaria. Smaller doses of the estrogens produced feminization of males, whereas larger doses also led to masculinization of genetic females. He suggested that estrogens might lead to cortical gonadal deterioration, but in view of the extreme adrenal hyperplasia, he suggested adrenal androgens as a factor and hence designated his observations as the "experimental adrenogenital syndrome." The presence of the pituitary is essential to the production of adrenal hyperplasia, but not necessarily to the production of genital effects. Although Witschi was unable to induce similar sexual alterations with stilbestrol, it is noteworthy that in Rana the adreno-

cortical hyperplasia does not occur with this compound. Little is known of the effects of large doses of stilbestrol on the human maternal or fetal adrenal cortex during pregnancy. Is it possible that temporary fetal or maternal adrenocortical hyperplasia with the production of androgens occurs under the influence of large doses of stilbestrol during pregnancy in selected instances?

Cases 1 and 4 were the most severe, and in these stilbestrol was administered earlier in the course of pregnancy. This suggests some relationship with the embryonic sequence of development of the genitalia as invoked for the effects of progesterone by Wilkins *et al.* (2). The effects described in our cases must be regarded as unusual. At the Joslin Clinic, 950 diabetic pregnant women have been treated with female sex hormones without any apparent influence on the genitalia of the offspring (L. Gillespie, personal communication); 700 were given stilbestrol, 25 mg. per day initially and eventually 125 mg. daily. Treatment was generally begun very early in gestation and continued until delivery. It must be inferred (if stilbestrol played a role in the patients described here), that only a rare fetus or pregnant woman is susceptible to estrogens in a manner leading to virilization. The nature of the susceptibility is unclear. Analogous contrary evidence exists for the culpability of certain progestins in effecting masculinization of the female infant as described by Wilkins *et al.* (2). Rakoff (personal communication) has employed these same hormones through the years without observing the effects described by others.

Although feminization of the male fetus by estrogens has been described in some species (5–7), it has not previously been reported in man. Recently, 1 such possible event in man has been called to our attention (13).

It should be added that so-called "non-adrenal" or "non-hormonal pseudohermaphrodism" has occasionally been described. We cannot incriminate estrogens in our 4 cases with finality at this time. On the other hand, cases previously assigned to the non-hormonal group have subsequently been associated with the use of progestins in one of our clinics (A.M.B.).

SUMMARY

Four instances of masculinization of the female infant, attendant upon the administration of stilbestrol to the mother during pregnancy, are described. No other causative factors could be recognized. The mothers received no other hormones, none was suffering from a virilizing syndrome, and the infants were excreting normal amounts of 17-ketosteroids. Such an occurrence in man is regarded as unusual in the face of the widespread use of estrogenic hormones during gestation, but it is *not* without its precedent in experimental embryology, in which field the paradoxical effects of estrogens on the female infant have been described. A peculiar and rare suscepti-

bility of the human adrenal to the action of stilbestrol is suggested. Experimental evidence points to adrenal hyperplasia consequent to the administration of estrogens as a possible factor, although a non-hormonal teratogenic action is not ruled out. It is expected that other similar cases of masculinization associated with the use of estrogens during pregnancy will be discovered.

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March 19, 1959

Addendum

Dr. Roy Hertz of the National Institutes of Health has observed clitoral enlargement in a female child who had ingested estrogen-contaminated vitamins, and rapidly subsiding clitoral hypertrophy in a newborn female whose mother had received stilbestrol during the pregnancy. Dr. Judson J. Van Wyk, University of North Carolina, reports to us that he has observed moderate clitoral hypertrophy and posterior fusion of the labioscrotal fold in an infant born of a mother who received micronized stilbestrol (25 mg. daily) beginning about the eleventh or twelfth week of gestation; he states that the treatment "was well toward the end of the period during which one would anticipate any anatomical effects on the urogenital sinus and would expect the defects to be mild."

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IDENTIFICATION OF "FREE" TETRAHYDROCORTISONE IN HUMAN PERIPHERAL PLASMA*

TO THE EDITOR:

In two previous reports (1, 2) we have suggested that even in certain "normal" persons much of the Porter-Silber reactive material detected in plasma may *not* be Δ^4-3-ketocorticosteroids, but may possibly be "free" tetrahydro E or tetrahydro F, and have cautioned against the uncritical acceptance that the plasma level of "free" Porter-Silber reactive material reflects the true state of adrenocortical activity. We are now presenting evidence that tetrahydro E in the unconjugated state is indeed present in "normal" human peripheral plasma.

To attain levels of corticosteroids in human plasma sufficiently high to permit isolation of individual compounds for definitive identification, 10 liters of fresh human bank plasma were processed. Upon preliminary assay of this plasma, the concentration of "free" Porter-Silber reactive material exceeded that of the "free" Δ^4-3-ketocorticosteroid content as measured by the isonicotinic acid hydrazide (INH) reaction by 5 micrograms (μg.) per 100 ml. This would result in a maximal potential expectancy of 500 micrograms of tetrahydro E or tetrahydro F in 10 liters of plasma.

* This work was supported in part by a grant from the U. S. Public Health Service, C-2383(C4).

The methodology described by us in a previous paper (3) was adopted throughout this isolation procedure, *i.e.*, protein precipitation followed by ethyl-acetate extraction and hexane:methanol:water partition. The various column chromatographic systems used included Florisil and silica gel as adsorbents, and chloroform, ethanol, toluene, cyclohexane and ethylene dichloride in various amounts and proportions as eluents. The gradient elution chromatographic procedure of Morris and Williams (4) was also used with certain modifications.

In addition, paper chromatography was employed in many cases for separation of interfering chromogens from steroidal materials; the modified Bush system as described by Eberlein and Bongiovanni (5) was the most useful among the many systems that were tried.

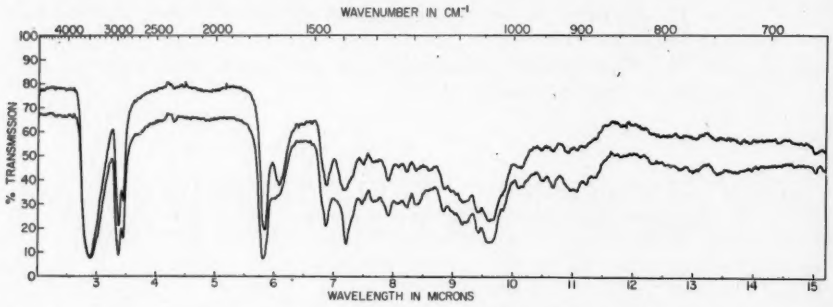

FIG. 1. Micro-infrared spectra in KBr. Top: Reference spectrum of tetrahydrocortisone (Merck & Co.). Bottom: Spectrum obtained from human peripheral plasma extract. Absorption band at 1650 (missing in spectrum #617 in Dobriner's Atlas) is due to KBr.

Apart from confirming by infrared spectroscopy and other criteria the previously claimed identification of hydrocortisone and corticosterone by several authors (6–8), we observed a highly polar fraction throughout the various column and paper chromatographic procedures. From reproducible Rf values observed on paper and the polarity as determined by solvent elution of silica-gel columns according to the method of Sweat (9) we became convinced that this fraction had to be tetrahydro E or tetrahydro F. Group reactions, *e.g.*, Porter-Silber (10), isonicotinic acid hydrazide (INH) (1) and blue tetrazolium reduction (11) yielded further evidence in favor of this choice of identity. We were finally able to obtain 280 μg. of a plasma fraction that was pure enough to submit a small quantity (eluted from a silica-gel column) to comparative infrared microspectroscopy. In Figure 1 the spectrum of an authentic sample of tetrahydro E (Merck & Co., Lot #55R1121) twice recrystallized from alcohol is compared with that of our isolated plasma fraction under identical experimental conditions. The sim-

ilarity of the two spectra would appear to leave little doubt as to the identification of our fraction as tetrahydro E. The quantities employed for these spectra were 18 and 19 μg., respectively. Micro KBr discs were prepared according to a technique developed by Dr. W. B. Mason (12). Technical details as well as further identification data concerning adrenocortical steroids in human peripheral plasma are now being prepared for publication.

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February 19, 1959

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The Endocrine Society

THE 1960 ANNUAL MEETING

The Forty-Second Annual Meeting of The Endocrine Society will be held in the Eden Roc Hotel, Miami Beach, Florida, Thursday, Friday and Saturday, *June 9, 10 and 11, 1960.*

The Chairman of Local Arrangements is Dr. Kenneth Savard.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 10, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

All members are urged to make their hotel reservations immediately. The Eden Roc will hold 250 bedrooms and the adjoining hotels, the Montmartre Hotel and Lucerne Hotel, will hold 75 rooms each for members until May 1, 1960, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations early, directly with the hotel, advising them of time and date of arrival and departure. If you plan on remaining through June 12 for the American Medical Association and other meetings, it is imperative that your reservation be made from June 12 on, directly through the A.M.A. Housing Bureau, c/o Miami Beach Convention Bureau, 1700 Washington Avenue, Miami Beach, Florida, since no reservation beyond June 12 can be accepted directly by the hotels. Make your reservations early and avoid disappointment. Hotel reservation card will be mailed to members shortly after January 1, 1960.

Final program, membership card and advance registration forms will be sent on May 1, 1960 to members whose current dues have been paid.

Those wishing to present papers, which will be strictly limited to ten minutes, should send an original and 4 copies of the title and abstract to the Vice-President, Dr. Robert H. Williams, University of Washington School of Medicine, Seattle 5, Washington, *not later than February 15, 1960.* It is imperative that the abstracts be informative and complete with results and conclusions—not a statement that these will be presented at the meeting—in order that they may be of reference value and suitable for printing in the program. The reading and processing of approximately 200 abstracts submitted each year and compiling of the program from these involves a tremendous amount of time and effort on the part of the

Program Committee. THE COUNCIL REQUESTS THAT AUTHORS ADHERE STRICTLY TO THE FOLLOWING RULES WHEN SUBMITTING ABSTRACTS; OTHERWISE THEY CANNOT BE CONSIDERED:

1. IT IS ASSUMED THAT ABSTRACTS SUBMITTED FOR THIS PROGRAM HAVE NOT AND WILL NOT BE SUBMITTED ELSEWHERE.
2. Abstracts may not exceed 200 words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formula cannot be used.
3. The title heading must be arranged as follows:
   Line 1. Title, not to exceed 15 words;
   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be written after his name.
   Line 3. Institution of origin and city in which institution is located.
4. Authors who wish receipt of their abstracts acknowledged should enclose self-addressed post card with the title of the abstract noted thereon.
5. Authors are also to enclose 3×5 inch filing cards, one for each author, giving his name and initials in the upper right-hand corner, and the complete title of the abstract with all the authors listed in the center of the card. There should be a second card enclosed listing the title, all authors, and the mailing address of the senior author. This is necessary for indexing. Senior authors will be notified of the acceptance of their papers for the final program.
6. The body of the abstract, typed double space, should follow the heading. The original copy should be on bond paper. The original and 4 copies should be forwarded to Dr. Williams.
7. Abstracts should be letter perfect, since there will be no opportunity for proof reading by the authors.

THE 1960 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

FRED CONRAD KOCH AWARD

In 1957 a substantial legacy was bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society. Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J. B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—

Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer; 1959—Dr. Gordon L. Farrell. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE UPJOHN SCHOLAR OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. A grant has been made available through the generosity of the Upjohn Company, and will be awarded to an established investigator or teacher in the field of endocrinology who wishes to extend the opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be

obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

ELEVENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE AND DEPARTMENT OF CONTINUING MEDICAL EDUCATION

SAN FRANCISCO, CALIFORNIA

November 9 through 13, 1959

For information concerning the program and registration, write to *Seymour M. Farber, M.D.*, Assistant Dean in charge of Continuing Medical Education, University of California Medical Center, San Francisco 22, California.

The registration fee is $100. Residents and Fellows will be admitted for a reduced fee of $30. Those wishing to reserve rooms should communicate directly with the Whitcomb Motor Hotel, 8th and Market St., San Francisco.

(For program, see the June issue of the Journal)

The American Goiter Association

THE 1960 ANNUAL MEETING

The next Annual Meeting of the American Goiter Association will be held in *London, England, July 5–9, 1960,* in the Great Hall of the Royal College of Surgeons. This meeting, co-sponsored with the London Thyroid Club, will be the Fourth International Goiter Conference. For further information write Dr. Selwyn Taylor, 3 Roedean Crescent, Roehampton, London, S.W.3, England.

JOHN C. MCCLINTOCK, *Secretary,*
149½ Washington Avenue,
Albany 10, N.Y.

SUBMISSION OF ABSTRACTS FOR THE PROGRAM OF THE FOURTH INTERNATIONAL GOITER CONFERENCE

Abstracts of American papers to be considered for presentation at the Fourth International Goiter Conference, London, England, July 5–9, 1960, should be sent to Dr. J. E. Rall, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda 14, Maryland. Abstracts from all other countries are to be sent to Dr. Selwyn Taylor, 3 Roedean Crescent, Roehampton, London, S W.3, England.

All American abstracts must be received by Dr. Rall *by December 1, 1959.* They should not exceed 400 words and should be submitted in quintuplicate. Presentation of papers will be limited to fifteen minutes and a copy of the final paper must be sent to the London Secretary two weeks prior to the meeting.

THE VAN METER PRIZE AWARD FOR 1960

The American Goiter Association again offers the Van Meter Prize Award of $300.00 to the essayist submitting the best manuscript of original and unpublished work concerning "Goiter—especially its basic cause." The studies so submitted may relate to any aspect of the thyroid gland in all of its functions in health and disease. The Award will be made at the Fourth International Goiter Conference in London, England, July 5–9, 1960, where a place on the program will be reserved for the winning essay-

ist if he can attend the meeting. For 1960, the recipient of the Award will receive consideration for an award of a travel honorarium.

The competing essays may cover either clinical or research investigations, should not exceed 3,000 words in length and must be presented in English. Duplicate typewritten copies, double spaced, should be sent to the Secretary, Dr. John C. McClintock, 149½ Washington Avenue, Albany 10, New York, *not later than January 1, 1960.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

BIOLOGICAL PHOTOGRAPHIC ASSOCIATION ANNUAL MEETING

Newly developed uses of medical and scientific photography will be the keynote at the 1959 Annual Meeting of the Biological Photographic Association. The meeting will take place *August 31 through September 3, 1959* at the Sheraton Mount Royal Hotel in Montreal, Canada. In the United States, information may be obtained from Carroll H. Weiss, 160 Riverside Drive, New York 24, N.Y.; and in Canada, from John L. Linder, 884 St. James Street W., Montreal 3, Quebec.

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Blood Groups. British Medical Bulletin, Volume 15, Number 2, May 1959. This symposium includes 14 papers covering work carried out by British researchers and reviews of recent contributions from various other countries. The subjects are: The relative importance of blood-group antibodies, inheritance of blood groups, biochemistry of human blood-group substances, antiglobulin reaction, hemolytic disease of the new-born, blood groups and disease, natural selection, anthropology and genetic linkage, blood-group substances in human tissues, adsorption of blood-group substances on to red cells, animal and human blood groups, and hemagglutinins in seeds. This volume should be of interest not only to the worker in the field of blood groups and blood transfusion, but also to the biochemist, immunologist, serologist, geneticist, anthropologist, pathologist, biologist, botanist, veterinarian and clinician (especially in pediatrics and obstetrics). The Medical Department, The British Council, 65 Davies Street, London, W.1, England. Price $3.25.

Carcinogenesis. Mechanisms of Action. Ciba Foundation Symposium, edited by G. E. W. WOLSTENHOLME, M.B., B.Ch. and M. O'CONNOR, B.A. The 29 members of the group, representing different disciplines and countries, discuss many aspects of cancer research including theories of carcinogenesis, viruses, co-carcinogens, hormones, pharmacological approaches, immunological aspects, antigens, protein-binding, biochemical mechanisms, leukemogenesis, the possible role of metals and of metal chelation, and problems of testing preparations for carcinogenic properties; 336 pages; 48 illustrations; 1959. Little, Brown and Company, Boston, Mass. Price, $9.50.

Clinical Evaluation of Neu Drugs. Edited by S. O. WAIFE, M.D. and ALVIN P. SHAPIRO, M.D. Fourteen authors present methods and standards by which new therapeutic agents can be appraised. The 16 chapters cover: Pharmacologic and other problems, from animals to man, experimental design and statistical problems, placebos and the evaluation of subjective response, the ethics of experimentation on human beings, the training of the investigator, clinical trials in infectious diseases, cardiovascular disorders, gastro-intestinal diseases, nutrition and metabolism, endocrinopathies and neuropsychiatric disorders, and problems of publication; 224 pages; 1959. Paul B. Hoeber, Inc., New York 16, N. Y. Price, $7.50.

Comparative Endocrinology. Proceedings of the Columbia University Symposium on Comparative Endocrinology held at Cold Spring Harbor, N. Y., May 25–29, 1958. Edited by AUBREY GORBMAN, Ph.D., Professor of Zoology, Barnard College, Columbia University, New York. One hundred and fifty experts from various countries took part in the Symposium, which covers: Endocrine factors in the adaptation and migration of fishes, regulation of migration and reproduction in birds, endocrine and behavioral factors in growth of mammalian populations, phylogenetic considerations, neuroendocrine substances, neuroendocrine mechanisms in control of the ovary, adrenal-hypophyseal interrelations, neuroendocrine control of TSH, pigmentary effectors, endocrinological aspects of protochordata, problems in comparative morphology and physiology of the vertebrate thyroid gland, renal thyroid tissue in fishes, the ultimobranchial body, thyroid gland function in cold-blooded vertebrates, thyroid-pituitary system, comparative anatomy of the hypophysis, species variation in protein and polypeptide hormones, effect of destruction of fetal hypophysis

upon sexual development, endocrine basis for reproductive adaptations, regulation of spermatogenesis in the frog, endocrine adaptations of estrous cycle and gestation, comparative physiology and biochemistry of the adrenal cortex and pancreas, morphology and physiology of the pancreatic islets, the caudal neurosecretory system of fishes, and observations on the corpuscles of Stannius; 746 pages; illustrations include electronmicrographs; 1959. John Wiley & Sons, Inc., New York 16, N. Y. Price $15.00.

Hormontherapie in der Frauenheilkunde. Grundlagen und Praxis. By Dr. med. JOACHIM UFER. A concentrated outline of hormone therapy in gynecologic disorders, including biochemistry and action of steroid and gonadotropic hormones from childhood to old age, therapeutic uses, diagnostic methods, and chapters on hormonal manifestations and treatment in various disorders of the reproductive tract; 158 pages; 66 illustrations; 1959. Walter de Gruyter & Co., Berlin, W.35, Germany. Price, DM 32.

Implantation of Ova. Memoirs of the Society for Endocrinology, No. 6. Proceedings of a Conference held at the Ciba Foundation, London, W.1, November 27, 1957. Edited on behalf of the Society for Endocrinology by P. ECKSTEIN. Eighteen participants discuss: The physiology of ovum implantation in mammals, the induction of pseudopregnancy and deciduomata in the rat by hypertonic solutions, delayed implantation in the badger, glycogen in early human implantation sites, a biochemical approach to the study of ovum implantation in the rabbit, the attachment cone of the guinea-pig blastocyst as observed by time-lapse phase-contrast cinematography, substances which inhibit pregnancy and the action of steroid hormones, the spacing of implantations in the mouse uterus, recent studies on hormonal control of delayed implantation and superimplantation in the rat, and histamine and the nidation of the ovum. The concept of the relative autonomy of the ovum within the uterus represents a significant contribution to existing knowledge; 100 pages; illustrated; 1959. Cambridge University Press, London; American Branch, 32 East 57th Street, New York 22, N. Y. Price $6.00.

(*The*) *Molecular Basis of Evolution.* By CHRISTIAN B. ANFINSEN, Ph.D., Chief of the Laboratory of Cellular Physiology and Metabolism at the National Heart Institute, National Institutes of Health, Bethesda, Maryland. A biochemical approach to the study of evolution considered as an integration of genetics and protein chemistry. The contents of the book include: The time scale and some evolutionary principles, genes as determinants of heredity, the chemical nature of genetic material, the substructure of genes, protein structure, the biologic activity of proteins in relation to structure, species variation in protein structure, genes as determinants of protein structure, the accuracy of protein synthesis, the biosynthesis of proteins, and a chapter on genes, proteins, and evolution. There are many new and controversial ideas to encourage further research; 228 pages; 99 figures; 1959. John Wiley & Sons, Inc., New York 16, N. Y. Price, $7.00.

(*The*) *Population Council. Annual Report for 1958.* Reports of the Executive Officers, outlining programs, grants and fellowships. Administrative Office, 230 Park Avenue, New York 17, N. Y.